

1973 - 74

*Program*

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INTERNATIONAL

POTATO



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***Program***

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***Conferences***

**1973 - 74**



SUMMARY  
OF  
CONTENTS

<u>SECTION</u>	<u>PAGE</u>
1 GERM-PLASM EXPLORATION AND TAXONOMY OF POTATOES	5
2 UTILIZATION OF GENETIC RESOURCES	49
3 LATE BLIGHT	139
4 BACTERIAL WILT	183
5 NEMATODE CONTROL	202
6 COLD HARDINESS	242
7 POTATO QUALITY	305
8 OUTREACH AND TRAINING PROGRAM	373

## FOREWORD

### THE INTERNATIONAL POTATO CENTER (CIP) APPROACH TO PROGRAM PLANNING

Dr. Richard L. Sawyer

CIP is a single-crop institution devoted to the improvement of the white potato (tuber-bearing *Solanums*) for the developing countries of the world. Its Core Program is funded by members of the Consultative Group on International Agricultural Research. First funding for active program development was received in 1972. The construction of facilities, staffing and initiation of research projects have been phased over the three-year period of 1972 through 1974.

In the development of CIP's program, the existing world expertise for potato improvement has been utilized in two basic ways. A continuing amount of major research effort with the potato has taken place for many years in North America and Europe. Through contracts, CIP is depending on this existing expertise for a portion of its research. CIP is also using the existing expertise in its International Planning Conference approach to long-term program development.

CIP's major program has been divided into ten major thrusts which include its research activities and the extension and training activities for developing countries. These are:

1. Systematic collection, classification, maintenance and distribution of all tuber-bearing *Solanum* species.
2. Utilization of the tuber-bearing *Solanums* to provide better adapted potatoes for developing countries.
3. Control of selected fungal pathogens.
4. Control of selected bacterial pathogens.
5. Control of selected viruses and insect vectors.
6. Control of selected nematode pests.
7. Development of potatoes with wider adaptation to environmental stress and insect pests.
8. Improvement of general nutritional quality, protein yield, and carbohydrate-protein balance in potatoes; the development of economical, scale-neutral methods of storage and processing for developing countries.
9. Seed production technology for developing countries; tissue culture for disease elimination, rapid multiplication and distribution of new clones.

10. Outreach Program (and affiliated Socio-Economic Projects) concerned with training personnel, the adaptation of research and the efficient distribution and utilization of the potato in developing countries.

In 1972, a five-year calendar of CIP events was established which called for an International Planning Conference approach to each major program thrust. Seven of these have presently been held and reports from these are included in this publication along with the general philosophy-strategy paper on outreach and training which was developed for and has been approved by CIP's Board of Trustees. The long-term calendar of CIP events calls for an international planning conference every three years for each major program thrust. A five-year plan is developed for the particular thrust involved at each planning conference.

The general planning conference strategy is to invite a team of up to twelve leading scientists in the world with the particular subject involved to meet for a one-week period with CIP scientists. In so far as possible, the participants represent both developed country technology and developing country needs. One month prior to the conference, a position paper is distributed to the participants to give them something to react to prior to the conference. The position paper gives the present level of technology for the particular subject involved and attempts to identify what needs to be discussed at the workshop. The position paper is written by one of the non-CIP participants who co-chairs the conference with CIP's Director of Research.

The participants at the conference have a mandate to develop a five-year plan of action for CIP activities within the particular field of work during the week of discussions. The five-year plan must include the identification of priorities. It must have a satisfactory balance of research activities between those requiring a long-term effort before breakthroughs can be anticipated and those requiring a short-term effort before a pay off takes place. The five-year plan for CIP activities takes into consideration the work being done or to be done at other research institutions. Thus, the CIP planning conference tends to have an impact on the program planning for research at many institutions and not just at CIP.

With such an approach to program planning, CIP management is attempting to utilize the best expertise that exists to help determine the research activities. CIP's major concern must be to improve the quantity and quality of the potato so that it is doing a better job for feeding the hungry people of the world.

In order to give balance to the planning conference approach, CIP's calendar of events calls for an overall external review each five years. The question of balance between thrusts and within thrusts will be addressed at this overall review. CIP is already developing the capability for an economic assessment amongst thrusts and for projects within a thrust which would take into consideration costs, potential pay off and developing country priorities.

1

***Germ-Plasm  
Exploration and  
Taxonomy  
of Potatoes***

## CONTENTS

	<u>Page</u>
I. Introduction	8
II. Germ - Plasm Exploration	9
III. Priorities for Germ-Plasm Exploration	10
IV. Taxonomy	14
V. Priorities for Taxonomic Research	15
VI. Summary of Priorities for Germ-Plasm Exploration	18
VII. Recommendations for Germ-Plasm Exploration	20
VIII. Recommendations for Taxonomic Research	20
IX. Collaboration suggested for Various Countries	21
X. Further Recommendations	22
XI. Suggested Time Schedules for Germ-Plasm Collections and related Activities.	24
APPENDIX 1	26
APPENDIX 2	36

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## I. INTRODUCTION

A wide genetic base for the breeding of new potatoes in all parts of the world needs no stressing. More and more, breeders are turning to the use of wild species and primitive cultivated forms to solve problems of disease resistance and adaptation to a wide range of environmental conditions.

Unfortunately, this reservoir of genetic variability, which until a few years ago had been taken so much for granted, is now diminishing at an alarming rate. The old highly complex pattern of diversity is, paradoxically, being replaced by the newly bred cultivars which are themselves derived from it. Such an erosion of genetic diversity is a process that must be halted, if breeders are to continue the production of new varieties now and in the near future.

The conservation of our rapidly diminishing germ-plasm resources in gene-banks, either by means of seed or in various other ways is becoming a matter of increased urgency year by year.

As a basis for genetic conservation, a carefully coordinated plan of exploration and collection needs to be established. It was the purpose of this workshop to examine the situation carefully in order to ascertain how much material already existed in potato gene-banks in various parts of the world and to make plans for collecting as much as possible of the genetic resources as yet unexplored.

At the same time, the workshop recognized the need for taxonomic research in order to provide a basis for genetic and plant breeding studies and to help with the more efficient utilization of the gene-bank material.

## II. GERM-PLASM EXPLORATION

Potatoes occur indigenously either as cultivated species or wild species, or both, right through the American continent, from the USA southwards to southern Chile and Argentina. Many collecting expeditions have taken place in the past, but much living material has been lost. In many areas the evidence of dried herbarium collections indicates the occurrence of a species where no living material has been gathered. Some species, which are known to widespread and variable, are represented in gene-banks by one or two collections only. Others are well-collected from a certain region but totally unrepresented by living material from the rest of their distribution area. Very often, the cultivated species have been collected haphazardly, without a properly coordinated plan.

It was therefore agreed that the first task of the Workshop should be to examine the priorities, country by country, for wild and cultivated species, for exploration work. Our task was greatly helped by the provision of a series of species distribution maps made by J. P. Hjerting, under the direction of P. R. Rowe; these gave details of living and non-living collections, species by species and country by country for the wild species held in the Sturgeon Bay gene-bank. The data of non-living collections were mostly taken from the literature. Unfortunately, there had not been time to make up similar maps for the cultivated species, but plans for these are under way and are noted in the list of recommendations made by this workshop.

A working document prepared for the workshop (see Appendix 1) formed an additional basis for discussion, as well as a report to F.A.O. on genetic erosion in potatoes (see Appendix 2).

Priorities were established under three heads:

1. Genetic erosion in progress or threatened.
2. Plant breeding needs, based on knowledge of the species and/or areas concerned.
3. Lack of living material in comparison with known distribution area, even though plant breeding needs are unknown. Taxonomic interest.

For each country or group of countries we have assigned A, B, or C priorities in descending order, A being most important. In some instances, the situation seemed of such importance or the threat of genetic erosion so great that an E category for emergency was assigned. This conforms to the classification used by F.A.O.

In a few cases the word none was assigned, where material was considered to be of no value or had already disappeared.

### III. PRIORITIES FOR GERM-PLASM EXPLORATION

#### 1. USA, Mexico, Guatemala

Little obvious genetic erosion is taking place with the wild species though they are of obvious interest to plant breeders because of their resistance to Phytophthora, viruses and insects. For many of the species, especially those of northern Mexico, and the USA, the living material in collections represents a very inadequate sample of the total geographical distribution. Although the main outlines of their formal classification have been worked out there are many biosystematic problems of interest that await solution.

There are no indigenous cultivated potatoes in the USA, but those of Mexico and Guatemala are so much threatened that the situation should be classed as "emergency".

#### 2. Central America

The wild species are of low priority for collection so far as erosion or plant breeding interests are concerned, but of slightly higher interest taxonomically, since they form a linkage group between Mexico and South America. There are, however, so few species and of such limited distribution that very little effort would be needed to collect them.

There would seem to be no indigenous cultivated species in Central America and the varieties grown are recent cultivars of no interest for germ-plasm exploration.

#### 3. Venezuela

We have some evidence of genetic erosion in the wild species and there is a complete lack of living collections in this group. We are not aware of any plant breeding needs in the wild species of Venezuela.

Erosion is also known in the cultivated species, and collections are apparently lacking in gene-banks, though there may be some in the Bogotá collection, Colección Central Colombiana. No information on plant breeders' needs is available.

#### 4. Colombia

Genetic erosion of the wild species is intense, because of habitat destruction (forest felling). No interest to plant breeders has yet been seen in this group, but more material is required in the living state.

Genetic erosion in the cultivated species is also intense, as a report from L. López indicates (see Appendix 2). One species (S. phureja) is of interest to breeders as a source of bacterial wilt resistance. Further collections are necessary, but later assessment of the situation will be made when the cultivated species distribution maps have been completed.

#### 5. Ecuador

The extent of genetic erosion of the wild species was not known to members of the workshop. Plant breeding needs were not yet apparent, but since practically nothing was available in living collections, this group would rate high on that count.

For cultivated species, C. Ochoa reported to other members of the workshop that genetic erosion was threatened and that very little material existed in gene-banks.

The cultivated material also seemed to be of considerable interest to breeders. Thus, on two counts at least, this group of potatoes was rated at highest priority.

#### 6. Perú

The wild species of Perú represent a particularly interesting part of the total number known, and from reports by C. Ochoa at the meeting and by Z. Huamán (see Appendix 2) they are threatened by erosion in a number of areas, both through the spread of urbanization and the destruction of forests.

In Ancash Department some of the wild and the cultivated forms may have suffered genetic erosion by earthquake damage. Furthermore, a rather limited amount of the wild species exists in collections and very little has been evaluated for plant breeding needs. Even so, what has already been examined shows considerable promise.

Considerable genetic erosion amongst the cultivated species was reported by C. Ochoa (see also Z. Huamán's report in Appendix 2), throughout the whole country, with the northern part of the country (Ancash to Piura) showing particular stress. In the central departments (Huánuco, Pasco, Junín, Huanca-velica) the threat is intense though not so much as in the north, where complete ranges of cultivars have disappeared in the last ten years. Even in the more traditional south, the newly bred cultivars such as Renacimiento are beginning to be accepted, but have not replaced the older ones as yet. However, it was felt that this situation would not last for long.

It was therefore agreed that an emergency classification should be assigned for genetic erosion, with A qualification both for plant breeding and taxonomic interests. Although much material had been collected by C. Ochoa, some of which was already in the CIP gene-bank, it was hoped that C. Ochoa's collections now growing at Cuzco could also be added and a central documentation file built up. This should be done with the greatest possible speed so that gaps in the collections could be identified and filled without delay. From the discussions it also emerged that the diploid, triploid and pentaploid forms were even more threatened than the tetraploids; hence from this point of view, and especially in relation to di-haploid breeding programmes and a study of the relationships between ploidy levels, in addition to the reasons discussed above, the workshop emphatically placed an emergency rating on the Peruvian cultivated potatoes as a whole.

## 7. Bolivia

From the personal knowledge of C. Ochoa and J. G. Hawkes, the Bolivian wild species were not thought to be in great danger of genetic erosion, but were rated more highly on plant breeding interest and lack of living collections (see also Appendix 1).

The cultivated species are threatened by erosion as Ochoa and Hawkes pointed out during the discussion (see also reports by M. Cárdenas and M. Zavaleta in Appendix 2). The category "B" should be assigned to them in respect of plant breeders' interests and need for further collection.

#### 8. Argentina

Wild species here have been well-collected in the past and are continuing to be explored by the Balcarce group (K. A. Okada). They are of great interest to breeders also for their Heterodera resistance, though the need for collections has been largely met. Therefore, the priorities here are not so high, even though it is certain that adequate collecting can be relied on from the Argentine colleagues.

The cultivated indigenous materials from Argentina are gravely threatened with extinction (see Appendix 1) and would rate an emergency category. More knowledge of these from a taxonomic view point is needed also.

#### 9. Chile

Leaving aside the non-tuberiferous Etuberosa series there is only one wild species in Chile - S. maglia. It would merit a low rating for erosion or plant breeding interest, but more collections, especially of diploid cytotypes, are needed from its whole range.

The situation regarding the cultivated Chilean potatoes has long since passed the emergency level and has reached the point of total extinction, according to a verbal report made by C. Ochoa at the workshop meeting, and a report to F.A.O. of which a copy was shown to the Chairman. All varieties now grown in Chile, with possibly one or two exceptions, are European or Chilean bred cultivars of recent origin.

Fortunately, C. Ochoa visited Chile in 1969 and made 270 samples, many of which are still kept in the University collection. There are also some 40 lines in the Sturgeon Bay gene-bank made by D. Correll. Although some may be duplicates there must still be a reasonable range of Chilean material available here and there, the major part being the extremely valuable Ochoa collection.

Since practically nothing now exists in the natural distribution area of the Chilean cultivated material the workshop could not assign priorities to this group, apart from the plant breeder's interest.

10. Uruguay, Paraguay, Brazil

A "B" priority was assigned to the wild species for collection needs. Little was known on genetic erosion or plant breeders' interest.

No indigenous cultivated potatoes exist in these countries.

#### IV. TAXONOMY

The pattern of variability of potatoes, as with most other ancient cultigens, is a complex one. Species boundaries are often unclear and phenotypic plasticity renders taxonomic problems even greater. Thus the cultivated forms have been separated into as many as 35 species or grouped together into a single one, S. tuberosum. In a similar way, the wild species, of which many are of great interest to breeders, are highly varied and can be grouped into about 150 species or split into more than 250.

From a practical point of view, and to be of maximum use to the breeders, a reasonably simple and workable taxonomic system will be of the greatest value. For this reason, a broad concept of species and grouping will provide a system in which fewer name changes are likely; even though it might not always fit in with current phylogenetic ideas.

The workshop considered that for the purposes under discussion, first priorities should be given to the provision of a simple and clear system of classification, special attention being paid to the cultivated forms.

At a different taxonomic level it would be of value to look at biosystematic problems at the interface between taxonomy and cyto-genetics, so as to provide further information about the possible evolutionary relationships between and within species. In this way, results of value to breeders, helping with the evaluation and utilization of material, should be forthcoming. Again,

this research should as far as possible be directed towards the cultivated species or wild species of interest and value in breeding research.

It was also suggested that numerical taxonomic projects might be carried out, using a programme already found to be valuable by D. Ugent for Mexican potato species.

The need for continuing to use chemotaxonomic methods when appropriate was also stressed.

## V. PRIORITIES FOR TAXONOMIC RESEARCH

This section should be read in conjunction with the Taxonomy section of the working document provided for the workshop (Appendix 1).

The taxonomic series into which the tuber-bearing species of the genus Solanum have been divided were examined in sequence, and priorities from "A" to "C" assigned for taxonomic research.

### Priorities

- |      |                         |
|------|-------------------------|
| None | 1. <u>Juglandifolia</u> |
| None | 2. <u>Etuberosa</u>     |

These two series have been traditionally assigned to the tuber-bearing group on their general morphology even though they are not tuber-bearing.

In view of the fact that no hybrids have been made between these and the tuber-bearing species, despite many attempts, no priorities for taxonomic research have been set on them, within the interests of CIP.

- |   |                         |
|---|-------------------------|
| C | 3. <u>Morelliformia</u> |
| C | 4. <u>Bulbocastana</u>  |

A satisfactory taxonomic system has been established for the species in these series. Some biosystematic work would be of interest.

- |   |                        |
|---|------------------------|
| C | 5. <u>Pinnatisecta</u> |
|---|------------------------|

(See notes in working document). There are still a few problems of species delimitation, through lack of living collections, but the main systematic outlines are clear.



- C. 6. Commersoniana (See notes in working document). The main taxonomic system in this series is reasonably clear.
- A few problems need elucidation, however.
- C 7. Circaeifolia No formal taxonomic problems exist in this series.
- A 8. Conicibaccata Basic taxonomic work, using living and dried collections, is urgently needed with this series. Biosystematic studies involving an analysis of the higher polyploids would also repay attention. Undoubtedly, several species could be sunk into synonymy.
- A 9. Piurana Much work is needed to define the limits of this series and to study the species in greater detail. More living collections are required.
- B 10. Acaulia Further taxonomic work is needed at the biosystematic level to clarify the status of subsp. aemulans and subsp. albicans, as the higher polyploids within subsp. punae.
- B 11. Demissa More biosystematic studies are needed on this economically important Mexican series (see working document).
- C 12. Longipedicellata The formal taxonomy of this group seems to be in a fairly satisfactory state, though some biosystematic problems would repay further study.
- C 13. Polyadenia (As for Longipedicellata).
- C 14. Cuneolata The relationship of the newly described species, S. anamatophilum, to S. infundibuliforme needs elucidation when living material of the former is obtained.
- B 15. Megistacroloba Studies on living material of certain species not as yet well known would be valuable.

- C 16. Ingaefolia . . . . Biosystematic studies are needed here,  
16a. Olmosiana . . . . but the taxonomy is satisfactory.
- 17a. Tuberosa . . . . Wild species (see working document).
- C Colombia . . . . Few problems.
- A Ecuador . . . . Much more research on this group is  
needed when living collections have been  
made.
- A Perú . . . . (As for Ecuador).
- A Bolivia . . . . (As for Ecuador).
- C Argentina . . . . Few problems.
- C Chile . . . . Few problems.
- E 17b. Tuberosa . . . . Cultivated species.

Apart from biosystematic studies on the individual species as mentioned in the working document (Appendix 1), there is an outstanding need for a series of booklets, describing the main group of varieties country by country, in a manner similar to those published by the Rockefeller Foundation and associates for the races of maize in Latin America.

Such a series of booklets should be written in collaboration with specialists in the various countries and should be published as soon as possible. C. Ochoa informed the workshop that he had already been engaged on such a study for Perú, and it was therefore hoped that the Peruvian booklet would be the first in the series. Each treatment would set out the data on levels of ploidy and/or species as well as being based on tuber, leaf, and flower characters, etc. Some keys to the identification of varietal groups might be possible, based perhaps on tuber and /or leaf and flower characters.

It was stressed that this whole subject should be treated as of "emergency" priority.

D. Ugent mentioned a scheme of studying potato fields in three or

four selected areas with a view to describing the agro-ecological and ethnobotanical aspects of "primitive" potato cultivation before it was swept away forever.

The workshop thought that this plan although of great interest, might fall within the area of the National Science Foundation for attracting support funds rather than CIP. Nevertheless, it was hoped that when a concrete plan was put forward, CIP would be given the opportunity of commenting on it with a view to providing certain on-the-spot facilities and general support.

## VI. SUMMARY OF PRIORITIES FOR GERM-PLASM EXPLORATION

### 1. U.S.A., México, Guatemala

#### Wild

1. C
2. A-B
3. A-B

#### Cultivated

1. E
2. C
3. C?

### 2. Central America

#### Wild

1. C
2. C
3. B

#### Cultivated

1. None
2. None
3. None

### 3. Venezuela

#### Wild

1. B
2. C
3. A

#### Cultivated

1. B
2. ?
3. A

### 4. Colombia

#### Wild

1. A
2. C
3. B

#### Cultivated

1. A
2. B
3. C?\*

\* Needs later assessment

Key on pages 14 and 15

5. Ecuador

Wild

1. ?
2. C
3. A

Cultivated

1. A
2. B
3. A

6. Perú

Wild

1. B
2. B
3. A

Cultivated

1. E
2. A
3. A

7. Bolivia

Wild

1. C
2. B
3. B

Cultivated

1. A
2. B
3. B

8. Argentina

Wild

1. C
2. B
3. C

Cultivated

1. A-E
2. C
3. A

9. Chile

Wild

1. C
2. C
3. B

Cultivated

1. None\*\*
2. C
3. None\*\*

10. Uruguay, Paraguay, Brazil

Wild

1. C?
2. C?
3. B

Cultivated

None

\*\* All gone; now only in collection.

Key: 1. Threat of genetic erosion.  
2. Plant breeders' needs.  
3. Lack of living material, taxonomic interest.

- A. High priority.
- B. Medium priority.
- C. Low priority.
- E. Emergency.
- None: No interest.

## VII. RECOMMENDATIONS FOR GERM-PLASM EXPLORATION

### A. Cultivated species

1. It is recommended that highest priority be given to exploration of cultivated potatoes in the following countries:

Perú, Ecuador, Mexico, and Argentina

2. The next highest priorities should be given to the cultivated potatoes of Venezuela, Colombia and Bolivia.

Notwithstanding the above, it is hoped that collecting activities will be carried out along a broad front in the next five-year period throughout the whole area under discussion.

### B. Wild species

1. The highest priority countries for wild species collections should be:

Venezuela, Colombia, Ecuador, Perú

2. Next highest:

Mexico, Bolivia

3. Lowest priority:

Central America, Argentina, Chile, Paraguay, Uruguay, Brazil.

## VIII. RECOMMENDATIONS FOR TAXONOMIC RESEARCH

It is recommended that the highest priority be given to taxonomic research on the following series:

- Conicibaccata  
Piurana  
Tuberosa From Ecuador, Perú and Bolivia (wild species).  
Tuberosa From all Andean countries (cultivated species).  
In view of their plant breeding importance, special emphasis should be placed on taxonomic research in this group.

#### IX. COLLABORATION SUGGESTED FOR VARIOUS COUNTRIES

1. U.S.A. Funds to be solicited to encompass wild species collections in 1973/1974; D. Ugent to make collections or report on other botanists willing to do so.
2. México, Guatemala D. Ugent to be responsible for cultivated species collections.
3. Central America Local contacts to be established e.g. Luis González at San José and Jorge Soria, at Turrialba, Costa Rica.  
Contacts to be sought in Honduras with the Ministry of Agriculture at Tegucigalpa and with Antonio Molina at the Escuela Agrícola Panamericana nearby.
4. Venezuela Contacts to be sought with Alvaro Montaldo and other colleagues at Maracay concerned with potato research.
5. Colombia Luis López at I.C.A. to be contacted and asked to put forward a planned collecting program.
6. Ecuador C. Ochoa to make contact with G. Albornoz, at Sta. Catalina (I.N.I.A.P.) and to arrange collaborative collecting expeditions.
7. Perú The problems of adequate germ-plasm collection are so large that it should be necessary, if possible, to have at least three collecting teams in the field at the same time, working to a coordinated network plan. This will need to be related to the material and documentation already existing in gene-banks, details of which were not available to the workshop. Help should be requested from Prof. C. Vargas of Cuzco.

8. Bolivia Further collecting work here will be made in collaboration with M. Zavaleta, M. Cárdenas and A. Vidaurre. Two Dutch expeditions will visit Perú and Bolivia in 1974 and 1975 respectively, and their routes and collecting programs should be coordinated into the general master plan. Support funds for the Bolivian collectors would be needed.
9. Argentina The main collecting work here will be done by K. A. Okada (I.N.T.A.). Priorities for the rapid collection of cultivated potatoes should be conveyed to I.N.T.A. with suggestions for immediate action. The two Dutch expeditions will also be working in this country, and plans should be integrated accordingly.
10. Chile Contacts should be made with:

Andrés Contreras	Valdivia
Alberto Cubillas	(now in Cornell)
Primo Accatino	(Santiago)
11. Uruguay It should be possible for K. A. Okada to collect here.
12. Paraguay "Miss E. Bordas" (Asunción) can be contracted and asked to send material from various parts of the country. Some modest support funds would probably be needed.
13. Brazil Contacts should be made with D. Mota da Costa, Raúl Ribeyro and O. Drummond.

Mota da Costa has already signified that he would be interested to make further collections in the southern states of Brazil.

#### X. FURTHER RECOMMENDATIONS

The workshop further recommends that:

1. A thorough survey should be made of all cultivated and wild material existing in gene-banks.

2. Mapping of cultivated material should be made by hand, in a similar way to the maps already made at Sturgeon Bay for the wild species.
3. A program should be put under way immediately in collaboration with Dr. David Rogers (Boulder, Colorado) to set all records into suitable form for computer storage and retrieval, using a system such as TAXIR\*. Key data should include accession numbers, collector's name and number, country, Department, Province, nearest locality, latitude, longitude, date, altitude, species name, etc. All other relevant information should be added. On the basis of this scheme, which falls into the international system agreed by F.A.O., printouts of basic material and computer distribution maps may be provided on request, and other data files concerning morphology, resistance to disease, etc. etc., may be later added. A pilot project is now under way using TAXIR in which CIP, Sturgeon Bay and the Commonwealth Potato Collection (C.P.C.) are collaborating, the results of which will be reported at the Rome meeting on the Conservation of Crop Genetic Resources in March.
4. First priorities in germ-plasm collecting should be given to cultivated forms, having in mind the general program already outlined.
5. Further help in germ-plasm exploration should be sought from competent persons other than those mentioned in Section IX such as J. P. Hjerting (Copenhagen).
6. Similarly, the first priorities in taxonomic research should be given to cultivated forms.
7. In relation to conservation work the workshop stressed that if the plan outlined in this paper should come to fruition that adequate facilities be made for the expanded amount of material which the gene-bank will have to hold.
8. Further, it was stressed that the bank should possess as broad a base as possible of material in the form of true seed. Research on the techniques to be used to convert the cultivated forms to true seed should be conducted as soon as possible.

\* "Taxonomic Information Retrieval System".



9. In addition, it was recognized that a limited number of cultivars, breeding lines and genetic stocks should be maintained clonally for a number of years, for demonstration and research purposes. Certain odd-number polyploids may also need to be maintained clonally.
10. Finally, the workshop suggested, although conceding that this was beyond its brief, that there was an urgent need to increase efforts in evaluation to facilitate the utilization of the material for the needs of developing countries.

## XI. SUGGESTED TIME SCHEDULES FOR GERM-PLASM COLLECTIONS AND RELATED ACTIVITIES

- 1973
1. Mapping of cultivated species (at Wisconsin or elsewhere).
  2. Collecting expedition in:

Peru	(C. Ochoa, Jackson, etc.)
Mexico	(Ugent)
Argentina	(Okada)
Bolivia	(Zavaleta, etc.)
Ecuador	(Ochoa, <u>et. al.</u> )
	(or in 1974)

Small funds to be set aside for collecting aid in areas of low priority.

- 1974
1. Collecting expeditions in:

Peru	(Ochoa, Jackson, Huamán)
Bolivia	(Zavaleta, etc.)
Colombia	(López, etc.)
Venezuela	(López, Montado)

First Dutch expedition to Peru, Bolivia, Argentina.

2. N. S. F. Program in Peru (Ugent)
3. Continue data storage program, computer mapping, etc. (Rowe; Rogers, Hawkes).

- 1975
1. Collecting expeditions in:

Peru	(CIP Staff)
Second Dutch expedition to Peru, Bolivia, Argentina.	
Colombia	(López <u>et. al.</u> )
Mexico	
  2. N. S. F. Program (continued) (Ugent)

3. Data storage programme (continued).
4. Junior fellowships (2) for germ-plasm and taxonomic studies.

- 1976
1. Collecting expeditions in:  
    Perú  
    Other countries as considered necessary.
  2. Workshop on Germ-Plasm  
    collection, classification, maintenance and utilization.
- 1977
1. More collecting efforts to be concentrated in areas not adequately covered in previous four years.
  2. Further action to be taken on the basis of the 1976 Workshop on Germ-Plasm collection, classification, maintenance and utilization.

Members of the workshop ended the meeting by expressing their thanks to Dr. Sawyer, Director General of CIP, for inviting them to take part in the meeting and for generously providing facilities and hospitality.

## APPENDIX 1

### Notes for the Planning Conference on Exploration and

### Taxonomy of Potato Species, at C.I.P., Lima,

January 1973

#### A. EXPLORATION

The extent to which potato exploration work is needed will not be known exactly until the survey of material in the Sturgeon Bay gene-bank is completed. We shall then be in a position to relate the material actually available to the total distribution area of each species and in this way identify gaps in the coverage. By January 1973, this information should be available, as well as data from C.I.P. itself, the C.P.C., C.C.C. and other collections.

At this stage, certain guidelines can be set out, as follows:

#### 1. U.S.A., México and Guatemala

- a) Wild species An important center of variability occurs in Central Mexico, with another center of lesser importance in Guatemala. North of the 26th parallel the species thin out and only two are to be found in the U.S.A. The approximate distribution of species in this area are known but living collections are almost certainly limited to a few easily accessible localities. Therefore, much collecting work in México and Guatemala is needed, related to what collections exist in Sturgeon Bay as well as the Rockefeller Foundation potato seed bank in Chapingo. The help of United States botanists should be enlisted to collect seeds of S. jamesii and S. fendleri from throughout their ranges in the southwestern States.
- b) Cultivated species There was probably never very much variation amongst the cultivated potatoes of México and Guatemala, and no indigenous varieties are known from the U.S.A. The variation that once existed in the Mexican and Guatemalan cultivars has now probably been largely if not entirely replaced

by bred cultivars of the type introduced by the Mexican Ministry of Agriculture, in association with the Rockefeller Foundation.

All types so far found in this region are 4x: S. tuberosum forms, many of them with characters of subsp. andigena. They undoubtedly sprang from post-Colombian introductions.

The present situation should be evaluated especially for the remoter higher altitude potato growing areas in Mexico and Guatemala where recent cultivars might not yet have become established.

## 2. Central America (Honduras, Nicaragua, Costa Rica, Panamá)

a) Wild species The amount of diversity in these countries is slight, being confined to a few species in series Conicibaccata. Living collections are needed, however, since most former collections have died out, and what remains is possibly one or two lines only of S. longiconicum from Costa Rica.

b) Cultivated species In Central America it seems probable that all forms cultivated belong to S. tuberosum subsp. tuberosum, recently introduced or bred locally. They are probably of little interest for gene bank purposes.

Help in this area for wild and cultivated collections might be solicited from I.I.C.A. at Turrialba, Costa Rica.

## 3. Venezuela, Colombia, Ecuador

a) Wild species In these countries the wild species still need to be collected, since very little living material is available, apart from certain collections of S. colombianum, S. flahaultii, S. tuquerrense and S. moscopanum.

Several species have never been available for study in the living state (see taxonomy section) and co-ordinated collecting plan for the whole area should be set up, with headquarters in Bogotá.

b) Cultivated species Collections have been made in Colombia, Venezuela and Ecuador by the writer and Colombian colleagues,

and have been cultivated in Bogotá in the Colección Central Colombiana. This took place some 20 years ago, and new collections should be made of diploid and tetraploid forms to replace losses and complete the collection.

#### 4. Perú

- a) Wild species This is the most important country in South America with regard to variability in wild and cultivated species. Many wild species collections have been made and are stored in gene-banks as living material. However, a vast amount of collecting still remains to be done in all parts of the country, based on the distribution data already obtained by Ochoa and others. This would seem to be top priority so far as the conservation of wild plant genetic resources is concerned.
- b) Cultivated species A well planned and co-ordinated scheme for the collection of cultivated species throughout the country is needed, related to the collections already present in C.I.P., Sturgeon Bay and elsewhere. Special attention should be paid to weed forms in southern Perú and varieties from the remoter areas where the new cultivars and selections have not yet penetrated. Attention should be paid also to the eastern slopes and valleys of the Andes to search for S. phureja cultivated material. The help of Professor C. Vargas and Ing. Agr. Fidel Flores in southern Perú should be engaged.

#### 5. Bolivia

- a) Wild species These have been collected recently, in 1971, by the Birmingham University Expedition and it is hoped to augment these collections by the Dutch expeditions in 1974 and 1975. Special attention is being paid to certain regions for the solution of taxonomic problems (see taxonomy section).
- b) Cultivated species Much exploration work is still required, even though the 1971 material is now conserved at C.I.P. The continued help of Ings. Agrs. M. Zavaleta, H. Gandarillas and S. Alandia will be needed, as well as advice from Professor Cárdenas and further help from Sr. Vidaurre of Potosí. A co-ordinated plan should be elaborated, involving help in the collection of wild and cultivated material by the colleagues mentioned above.

6. Argentina

- a) Wild species Much collecting work has been done by Hawkes and Hjerting, and is being ably continued by Okada. The taxonomic and genetic conservation problems are well understood on the whole, though certain areas in the east, west-central and north-east regions must still be explored.
- b) Cultivated species The collections made by Hjerting and co-workers in the 50's have now been lost and may not now be replaceable. Material might be obtainable from Brucher, though this is doubtful in view of possible virus infections and poor documentation. A search should be made without delay for cultivated and weed forms, especially since Brucher has claimed that material of different ploidy level exists in Argentina. Help should be obtained from Okada and also Virsoo (Tucumán) if he is able to take part in this project. The assistance of Ruis Leal in Mendoza should also be enlisted for surveys in Mendoza, San Juan and the Nahual Huapi regions, where more collecting is certainly needed.

7. Chile

- a) Wild species Very few tuber-bearing wild species occur in Chile. Collections of the non-tuber-bearing series Etuberosa would be valuable from the botanical point of view, since although no crosses with the Tuberosa gene pool have yet been accomplished there is no reason to suppose that this could not be accomplished in the future.
- b) Cultivated species The very valuable Ochoa collection from southern Chile will be conserved and discussions should take place between Ing. Ochoa and Chilean agronomists to see whether there is any other material that ought still to be collected.

8. Brazil, Paraguay, Uruguay

No indigenous cultivated forms are known from these countries but living collections of S. commersonii, S. chacoense subsp. muelleri and S. caldasii are needed, especially in the form of fertile diploids.

## B. TAXONOMY

Suggestions for future taxonomic work are set out series by series.

### 1. Juglandifolia

Living collections and taxonomic work on species in this series are needed, but this hardly seems to fall within the interests of C.I.P. Discussions next August at the special interest group on Solanaceae during the Boulder meeting on Systematic and Evolutionary Biology might well provide decisions on future work in this series and its relationship to other genera (e.g. Lycopersicon, etc.)

### 2. Etuberosa

Taxonomic work, based on living collections, is needed for this series, though probably not at a very high level of priority in view of the so far insurmountable crossability barriers between it and the tuber-bearing species. It remains of interest, however, in view of the immunity to viruses of the species so far investigated. Hybrids may perhaps be obtained in the future through naked protoplast fusion, so the series should perhaps not be totally disregarded in C.I.P. programmes.

### 3. & 4. Morelliformia and Bulbocastana

The main outlines of the species in these series are clear so far as formal systematics is concerned. Experimental work on species relationships, however, may well provide information of value to breeders in the future.

### 5. Pinnatisecta (including Trifida)

The formal taxonomy of this group, also, remains reasonably clear, though S. hintonii and S. nayaritense have not yet been collected in the living state and are thus urgently in need of investigation. Natural and artificial hybrids between species in Pinnatisecta, Bulbocastana and Morelliformia need investigation also. The hybrid nature of S. x michoacanum as postulated by Correll, is now accepted, and S. trifidum is recognized as a genuine species of considerable interest. More collections and taxonomic studies are needed with S. stenophyllidium. The nature of S. nicaraguense has now been

postulated and perhaps needs little further attention.

6. Commersoniana

The taxonomy of this series is fairly well-known in main outline. However, some investigations on the interface between the two subspecies of S. commersonii in Entre Ríos province are needed, as well as the forms of S. chacoense subsp. muelleri in southern Brazil. Much more knowledge of the taxonomic position and relationships of S. calvescens is required, and for this purpose more collections should be made in Minas Gerais, Paraná, Santa Catarina and Rio Grande do Sul States.

S. yungasense and S. tarijense are well-known, though the origin of the former would repay investigation.

7. Circaeifolia

Relationships between the two species in this series, S. capsicibaccatum and S. circaeifolium, should be investigated, as well as their position vis-a-vis other series.

8. Conicibaccata

Much taxonomic work is urgently needed on this series, since many species are unknown in the living state and others are not well studied. Some species boundaries need revision and cytological work on the nature of the polyploid series is required. An interesting chemotaxonomic study of this series is being carried out by L. López (Colombia).

9. Piurana

Since my 1963 "Revision" was published I have become less happy about this series. If it is retained some species will need to be removed from it and placed in series Conicibaccata or Tuberosa. Much material needs to be assembled in the living state and biosystematic studies carried out. Polyploidy is also seen here and needs further cytological investigation.

10. Acaulia

Whilst the taxonomy of subsp. acaule and subsp. punae is clear,



much still remains to be understood with regard to subsp. albicans and subsp. aemulans. Higher polyploids of subsp. punae in northern Perú should be further studied as well as the origin of this apparently autotetraploid species S. acaule, as a whole.

11. Demissa

This is a rather heterogeneous group, mainly of hexaploids, but with two pentaploids and a diploid species, S. verrucosum. This latter should perhaps be restored to series Tuberosa where Rydberg first placed it. Of the two pentaploid species, the origin of S. edinense has been verified by Ugent, but that of S. semidemissum is unknown. One common ancestor for the hexaploids (S. verrucosum) has been postulated by Marks but the other parents for each are completely unknown.

The whole group would form the basis for an excellent cytotaxonomic study.

12. Longipedicellata

Species boundaries for the taxa in this series are satisfactory, though S. stoloniferum may need further subdivision. The nature of S. vallis-mexici has been established by Marks, and species relationships among the tetraploids have been established by the writer. However, the parental diploids of these polyploids are not known, and cyto-genetical work is therefore needed to elucidate this problem.

13. Polyadenia

The biosystematic relationship of this series to others should be further investigated. More should be known about the distribution of S. lesteri through new collections in southern México (Oaxaca, etc.)

14. Cuneoalata

There seems to be no outstanding taxonomic problems in this series. (But a recently described Peruvian species may be placed here).

15. Megistacroloba

Several species in this series are imperfectly known, and some have never been studied in the living state.

16. Ingaefolia

The relationship of the species S. ingaefolium and S. rachialatum to each other and to S. olmosense in series Olmosiana needs investigation, and their distribution areas should be defined more precisely.

16a. Olmosiana

See under 16, above.

17. Tuberosa

- a). Wild species Because so many species are placed in this series, it will be convenient to discuss them by countries.

Colombia Only two species are known for this country, one of which, S. lobbianum, has never been studied in the living state.

Ecuador There are probably about four species in this country. None is well known experimentally, and more work on all of them is required.

Perú There is an extreme richness of Tuberosa species in Perú, greater than in any other part of the Andes, with some thirty species now recognized. Much work is needed on a wide range of species from this country, and it seems probable that greatest efforts should be concentrated on various systematic and evolutionary problems on the Peruvian taxa.

Bolivia Some fifteen species are now recognized from Bolivia, one or two of which spread into Perú. Investigations on several groups of Bolivian species are under way at Birmingham (especially the weed species group which also spreads into Perú), but work by other investigators would be welcomed.

Argentina The taxonomic problems of most Argentinian Tuberosa species are solved; but with some work still needed on certain taxa which spread into Bolivia.

Chile Only one species (S. maglia) is known, and little work is needed.

17a. Cultivated species

- (i) S. ajanhuiri The nature and origin of this species is being studied by Z. Huamán.
- (ii) S. stenotomum This species needs further taxonomic study with regard to infraspecific classification. Since it is generally assumed to be the most primitive of all the cultivated potato species, biosystematic studies are required to identify its wild prototype.
- (iii) S. phureja The relationship of this diploid species to S. stenotomum should be further investigated, as well as the forms of this species from Perú, if they can be found.
- (iv) S. x chaucha Cyto-taxonomic studies on this hybridogenic species are being carried out by M. Jackson to investigate the extent of gene flow from diploid to tetraploid cultivars and vice-versa. Artificial lines are being synthesized and compared with naturally-occurring lines.
- (v)&(vi) S. x juzepczukii and S. x curtilobum The nature of these two hybridogenic species has already been established (Hawkes, 1962).
- (vii) S. tuberosum (including subsp. andigena). Good evidence has now been provided from recent studies (unpublished) by P. Cribb to substantiate the hypothesis (Hawkes, 1956) of its origin from natural crosses of S. stenotomum x S. sparsipilum. A paper by H. W. Howard also adds considerable weight to this hypothesis. Further work with subsp. andigena dihaploids is under way at Birmingham.

Classification of groups of varieties of subsp. andigena in the Andes would probably be worthwhile. Such a classification (following the lead of the excellent work of the maize geneticists) should include dichotomous keys and should be based on leaf, flower and tuber characters.

Final Conclusions

The preceding notes are set out as a basis for discussion in an attempt to show very approximately the areas where further efforts should be concentrated.

In the writer's view, emphasis should be placed on experimental studies linked to morpho-geographical data wherever possible. Cytology, genetics, chemo-and sero-taxonomy should be brought in, and numerical-taxonomic studies should be considered wherever possible.

From the results of the taxonomic studies it should then be possible to make the material more available for potato breeding and general utilization.

J. G. Hawkes,  
Birmingham.  
January, 1973

## APPENDIX 2

### Potato Genetic Erosion Survey - Preliminary Report.

January, 1973

J. G. Hawkes

#### 1. Introduction

The F.A.O. Unit for Crop Ecology and Genetic Resources asked me to carry out a survey, through correspondents, of the extent to which genetic erosion is or may be taking place in the centers of variability of the cultivated potato and its wild relatives.

Until 1971, I had assumed that very little erosion was taking place in the main gene centers of the South American Andes, though much evidence was available to show that in Chile the old land races had been disappearing rapidly from about 1950 onwards, because of the bad Phytophthora epidemics which occurred at that time.

However, whilst taking part in a collecting expedition to Perú and Bolivia in 1971, I became aware that the richness of varietal diversity had diminished very startlingly, as compared with the situation in 1939 when I last visited those countries to collect cultivated potatoes. I had assumed that the Andean potatoes would be protected from genetic erosion by the fact that standard European and North American varieties cannot be grown at the high altitudes to which the Andean potatoes are adapted. Nevertheless, the Andean potatoes themselves had changed. In fact, during this 30-year period the local breeders, some of whom I myself had helped to train, had begun the processes of breeding and selection which are now causing the replacement of much of the old richness of primitive forms and species by better yielding standard varieties.

Furthermore, agronomists and extension officers had promoted the cultivation of a limited number of selected variants or land races, even where new cultivars were not available. These also, were replacing the old richness of varietal diversity.

Fields were much tidier in 1971 as contrasted with 1939. There was an almost total absence of wild and weeds forms in the furrows and around the field borders, in marked contrast to their relative abundance in 1939. This indicated an apparent end to the process of co-evolution of crop and weeds by hybridization and gene flow from one to the other--a process which had probably been continuing for several thousand years, ever since potatoes were first domesticated.

Most expeditions to a gene center, our 1971 one included, tend to keep chiefly to the main roads, so that the desired areas may be explored in the time available. Hence the situation may be less alarming than appears at first sight. It is possible that in the more remote regions where the communications are poor, where the plant explorers or extension officers fail to penetrate, or where the stronger forces of tradition militate against the adoption of newer varieties, then the original genetic diversity may still remain intact. However, we can have little cause for complacency, since experience with other crops and in other parts of the world has shown that new high yielding cultivars can extend rapidly in a few years into extremely remote regions, thus displacing the old land races entirely.

So far as wild species of potato are concerned, the situation is somewhat better. The greatest threat is to the potato weed species, which, strangely enough, seem often to "take refuge" in maize or other fields at the lower altitudes, but cannot do so at levels where maize is not grown. The main threat to the truly wild species is the usual one of habitat destruction, particularly in the vicinity of the ever-expanding capital cities and in the natural forests, which themselves are threatened with complete destruction in many regions.

In view of what has been said above it seemed to be a matter of urgent necessity to attempt a more careful survey of genetic erosion in potatoes.

I discussed the matter with Sir Otto Frankel of C.S.I.R.O., Canberra, Australia, and Dr. Jorge León, Chief of the Crop Ecology and Genetic Resources Unit at F.A.O., Rome. They advised me to carry out a survey by correspondence with colleagues in those countries where the potato is cultivated or occurs wild, within the general area of its "natural" distribution, so as to obtain further information on the problem. It was further hoped that the information would be of value to Dr. Richard Sawyer, Director of the International Potato Center at

Lima, Perú, in helping him to plan a strategy for the exploration and conservation of potato materials in the gene-bank that is now being established in his Institute. The matter has also been discussed with Dr. Roger Rowe, Director of the Inter-regional Potato Introduction Station at Sturgeon Bay, Wisconsin, U. S. A., who has on my suggestion instituted a careful study of the genetic range of living material, species by species, in the Sturgeon Bay gene bank.

It must be emphasized that the present account is no more than a preliminary survey, which it is hoped will act as a basis and a stimulus for a more exact report at a later date. Enquiries were made from sixteen colleagues in México, Venezuela, Colombia, Ecuador, Perú, Bolivia, Argentina and Chile. Replies were received from five colleagues only, though fortunately these were from the main potato-growing countries of Venezuela, Colombia, Perú, and Bolivia. This account, then, represents the views and experience of these colleagues, as well as my own, and is thus incomplete. When the exploration work which will be planned and co-ordinated with the International Potato Center begins to take shape, the picture will undoubtedly become clearer.

## 2. Genetic erosion in the U.S.A., México, and Guatemala

- a) Wild species An important center of variability occurs in central México, with another one of lesser importance in Guatemala. North of the 26th parallel the species thin out, and only two are to be found in the U.S.A. So far as I am aware, there is no serious threat to the wild species in this area, though I should point out that I have received no information from correspondents and my last major expedition there took place in 1958.
- b) Cultivated species There was probably never very much variation amongst the cultivated potatoes of México and Guatemala, and no indigenous varieties are known from the U.S.A. The variation that once existed in the Mexican and Guatemalan land races has now probably been largely if not entirely replaced by bred cultivars of the type introduced by the Mexican Ministry of Agriculture, in association with the Rockefeller Foundation. A survey of the present situation is urgently needed.

## 3. Genetic erosion in Central America (Honduras, Nicaragua, Costa Rica, Panamá)

- a) Wild species The amount of diversity in these countries is slight,

being confined to a few species in series Conicibaccata. I suspect that habitat destruction in the high mountain forests constitutes a threat to the species growing in them, but I am not at present in a position to make a clear statement in this respect, since I have no potato-scientist contacts there and have not made a recent visit.

- b) Cultivated species In Central America it seems probable that all forms cultivated belong to S. tuberosum subsp. tuberosum, recently introduced or bred locally. They are probably of little interest for gene-bank purposes.

#### 4. Genetic erosion in Venezuela

- a) Wild species I have no knowledge of the threat to wild potato species in this country.

- b) Cultivated species Dr. Alvaro Montaldo writes:

"Venezuela cultivates 14,000 hectares of potatoes. Of these, 10,000 hectares are localized in the lower zone (450-1000 m) and the intermediate zone (1000-2000 m), where new varieties such as Sebago, Red Pontiac and Alpha are grown. The andean States of Mérida, Táchira and Trujillo at heights of between 2000 and 3500 m some 4000 hectares of potatoes are grown. About half of this area is occupied by old varieties such as Arbolona negra (S. andigena) and a very small part by S. rybinii ( $\approx$  S. phureja)".

Only the S. tuberosum subsp. andigena and the S. phureja forms need concern us here, and unfortunately the information is not detailed enough to indicate whether genetic erosion is taking place or not. Further information has been requested.

#### 5. Genetic erosion in Colombia

- a) Wild species I have no recent information but I suspect that the destruction of the forests which was taking place in 1948-51 when I worked in Colombia is still continuing, with the consequent diminution of the distribution areas of the wild species growing in them.



b) Cultivated species Sr. Luis López writes:

"It is difficult for me or for any of my countrymen to give good information on the extent of the erosion of genetic variability in cultivated potatoes in my country due to the lack of recent collections to compare with those that you yourself made in the years 1948-1951.

It is not difficult to imagine the loss of many cultivated varieties which are highly susceptible to diseases, bad shape and poor commercial quality since several highly selected hybrids have been distributed to the potato areas with good acceptance by growers and in the markets. Those newly bred cultivars such as 'Ica Purace', 'Ica Guantiva', 'Ica Tolima', 'Cumanday', etc. which are high yielding are replacing most of the old varieties in the whole country. The "Instituto Colombiano Agropecuario" through the Extension Service and the "Programa Nacional de Tuberosas" is encouraging the farmers to grow bred cultivars which have good yield, and resistance to certain diseases.

The demonstration to farmers of the advantages of growing bred cultivars in their own lands is convincing them that they should replace their old varieties with newly bred ones because they can achieve higher incomes. The highly selected hybrid 'Ica Purace' is actually cultivated in all the potato areas and more or less 30 per cent of the whole production of potato in the country is 'Ica Purace'. Nevertheless, consumers are very conservative and they are asking for the old commercial varieties such as 'Tuquerreña' in the departments of Boyacá, Cundinamarca and Nariño; 'Tocana Blanca', 'Tocana Rosada', 'Pamba Blanca', 'Pana Azul' in the department of Nariño; 'Yema de Huevo' (Solanum phureja) in most of the country, 'Salentuna' in Caldas and Antioquia departments. The extent of this requirement stimulates the farmers to grow them, despite their low yield.

Old varieties such as 'Lisarza' and 'Salentuna' in Caldas are impossible to replace because of their very good adaptation to high altitudes and resistance to transport damage on mules or car and so far no bred cultivar can compete with them in that respect".

It seems quite clear from this account that genetic erosion has taken place to a very large extent in Colombia;

nevertheless, the situation would seem to be by no means hopeless, especially since the will and the means to make new collections are present."

6. Genetic erosion in Ecuador

No information has so far been forthcoming from that country. The situation would probably be rather similar to that in Colombia.

7. Genetic erosion in Perú

a) Wild species Sr. Zósimo Huamán writes:

"In April 1971, during the Birmingham University Potato Collecting Expedition to Bolivia and Perú, we collected wild potato species in the Dept. of Puno, Cuzco, Apurímac and Junín, in the same places as I collected them in 1970 or in the type localities of Hawkes', Vargas' and Ochoa's species. For example, S. canasense, S. soukupii, S. multidissectum, S. raphanifolium, S. lignicaule, S. marinasense, S. calcense, S. ochoae, S. pumilum, S. bukasovii, S. pampasense, S. abbotianum; etc.

Some species such as S. hawkesii (Machu Picchu, Cuzco) S. longimucronatum (Curahuasi, Abancay, Apurímac), etc. are not found any more in their type localities.

The situation of wild species from the Lomas along the Pacific Coast is seriously threatened by the grazing of goats and use of desert land for building new towns. Thus, S. wittmackii in the Lomas de Amancaes, near Lima; S. newweberbaueri, in the Lomas del Cerro Morro Solar, Chorrillos, Lima, etc.

So far, I have not made collections of wild or cultivated potatoes in the Northern Region of Perú."

My own observations coincide with those of Sr. Huamán; in addition, my experience in Northern Perú indicates that certain species inhabiting medium altitude forests on the eastern slopes are threatened because of the threat to these forests themselves.

b) Cultivated species Sr. Zósimo Huamán writes:

"The Peruvian Ministry of Agriculture through its National

Potato Project has established Agencies for an Agricultural Extension Service all over the potato growing areas of Perú. This organization has at least one agency in each Distrito and its role is to encourage the growing of newly bred varieties, use of fertilizers, weeding practices, etc., by the Andean farmers in order to raise the national average yield of potatoes. Moreover, the recent Law of Land Reform and Law of Agricultural Co-operatives are giving more backing to crop improvement of potatoes, maize, etc.

All these organizations have already shown their effectiveness in the attempt to raise the standard of living of the Andean people. In this way the cultivated area with bred varieties such as 'Renacimiento', 'Mantaro', 'Mariva', etc. is increasing every year. Furthermore, there is a trend to grow some old cultivars which have a great acceptance in the market because of their quality, flavour, etc. for example, 'Ccompis' in Cuzco; 'Yurac sisa' in Apurímac; 'Chata Blanca' in Junín, etc.

The Peruvian Potato Programme has devoted great attention to its branches in Cuzco and Puno because here the level of education of the people retards the process of changing patterns of communal life and customs. Therefore, the replacement of old cultivars and primitive forms of potatoes is taking place very quickly in the center of diversity of potatoes. This is so not only in farms near the main roads, but also in small villages, through the activities of the Agricultural Schools or primary schools. It is quite common for teachers to introduce newly bred varieties and cultivate them in small patches near the school to demonstrate to the people their resistance to diseases, better yield, etc., compared to native varieties.

In the Central and North Regions of Perú, the depletion in variability of potatoes is even greater than in the South because the level of education has made the introduction of newly bred varieties easier.

In 1970 an earthquake of great intensity was concentrated in the Callejón de Huaylas, Ancash Department; consequently a large number of old cultivars of potatoes were lost because great extensions of cultivated land were covered by earth. Although a fortnight before the earthquake, Srs. Fermín de la Puente and Luis López collected almost 100 clones in markets

and fields near the main road, it is highly likely that a great deal of variability was lost. Therefore, it would be advisable to initiate collections on a large scale in those regions threatened by natural disasters.

In 1970, López and I took part in a collecting expedition around Cuzco, Apurímac, Ayacucho, Huancavelica, Junín, Pasco and Huánuco. We were able to appreciate that many varieties were not found in local markets because either they could not be sold easily or they were cultivated in small areas for family consumption. We made good collections in fields during harvesting and in the temporary heaps set out in the fields before storage.

Unfortunately, I have not made collections of cultivated potatoes in these Departments again and therefore I cannot give a precise report on the genetic erosion in potato germ-plasm which is taking place in those regions.

Some varieties of Solanum x curtilobum and S. x juzepczukii, which are cultivated around 4,000 m are highly susceptible to Spongospora subterranea, Synchytrium endobioticum, etc. In Huancavelica, Ayacucho and Puno I saw these heavily infected varieties being harvested and learned that the farmers were willing to change to the newer bred varieties grown by more prosperous local farmers which do not show a marked degree of infection.

One of the most threatened species is S. stenotomum subsp. goniocalyx in the North of Perú. Varieties such as 'Amarilla', 'Yema de Huevo', etc. are practically disappearing because they are highly susceptible to virus diseases and have a low yield despite their good quality."

The account given by Sr. Huamán speaks for itself. Genetic erosion is clearly taking place in Perú, and to an extent as great as that in Colombia. More details are required, though the general outline is clear. A well-planned and coordinated scheme for the collection of cultivated potato species throughout the country is needed, paying special attention to weed forms in southern Perú and to areas where the new cultivars and selections have not yet penetrated.

## 8. Genetic erosion in Bolivia

### a) Wild species Sr. Moisés Zavaleta writes:

"In weed and wild potatoes collected in different places in

Bolivia and at different times, we observed strong genetic erosion. Thus, in Mocomoco, Prov. Muñecas, Dept. La Paz, we found in 1958 five different kinds of 'taihua' a weed potato that grows like 'lelekoyo'; in maize fields; the tuber shape was either rounded or long and the skin colour was black, white, red or yellow. The tubers were about 120 grams each and some clones showed short dormancy. Recently we went to make a new collection of this material but could find only the red round-tubered form.

In Killumblaya, near Puerto Acosta, Prov. Camacho, Dept. La Paz we found another weed potato with same name, 'taihua', but this sample was different from that found in Mocomoco, and is very similar to one in Santiago de Huata, Prov. Omasuyos, Dept. La Paz.

In Culpina, Prov. Sud Cinti, Dept. Chuquisaca, we saw in 1964 many weed potatoes in potato fields, but when we returned in 1967 we observed few weed plants in the potato fields.

Our first collection of the wild species, S. achacachense was made from plants growing between stones by the road from Achacachi to Sorata in 1958. Today they can only be found on rocky slopes in places where they are inaccessible to grazing animals.

In 1963, going by road from Aiquile to Sucre, we collected many samples in the field between stones and scrub. In our 1969 trip we looked for the same samples but could not find any wild potatoes. However, far from the road amongst cactus and spiny scrub, we found some, because in these places they were protected from goats.

Thus erosion of genetic variability in weed and wild potatoes seems to be due principally to the elimination of weeds from fields of potato and to the grazing of animals such as goats which eat all wild potatoes, unless they are especially protected".

b) Cultivated species Professor Martín Cárdenas writes:

"The task to be undertaken is of course very immense since we do not possess enough information from surveys

some 30 to 40 years ago to tell what species have become extinct. A unique factor which menaced wild, weed and cultivated potatoes was the Reforma Agraria; a political law which has given all the available agricultural land to the Indians. Before this Reforma, landlords or patronos, having a special knowledge of certain fine potato varieties, had as many of these cultivated as possible. I still remember when visiting the high-altitude estancias a very fine assortment of 'Papa huaycus' belonging to several varieties of S. andigena and S. stenotomum. Now these are not to be found. I believe that it will be necessary to collect again as much as possible of the potato species and varieties, wild, weed and cultivated, and to keep it in a gene-bank before it disappears completely, through the destructive action of civilization".

Sr. Moisés Zavaleta writes on the cultivated potatoes, as follows:

"During the last ten years I have made many trips around Bolivia, collecting cultivated potatoes in many parts of the country.

In S. phureja it seems that most cultivars are infected with virus diseases, and for this reason farmers prefer to grow them on the upper parts of the mountains where sometimes there is a danger of frost.

In Millipaya, Prov. Larecaja Dept. La Paz, I found in 1962 great variability in S. phureja cultivars. The most important cultivars were 'Kellu phureja' and 'Chiar phureja'. In 1967, I returned to collect samples but could find none, even though I made enquiries of many farmers and walked to many farms to try and get samples of these two cultivars. During this collecting session I found a large field of 'Alka phureja' which is the most commonly grown cultivar of S. phureja.

Between Sorata and Tacacoma, 10 years ago I found many fields of 'Janko Phureja'; today it is difficult to find samples of these cultivars.

Ten years ago during my first trip to Tacacoma, I collected many samples of potatoes, most of them S. andigena.

This material was cultivated in glasshouses at the Belen Experimental Station and was also planted in the field. All samples showed good healthy plants, comparatively free from virus. In material collected subsequently in the same place most of the cultivars were seriously infected with virus diseases. I also found different varieties to those collected during the first visit, two of the most interesting being 'Pichuya' and 'Pala'.

In 1962 the S. ajanhuiri cultivars 'Janko ajahuiri' and 'Yari' were found in many parts of the Prov. Pacajes, Dept. La Paz. However, in my 1968 trip I found few samples, most of them with very small tubers.

Many cultivars of S. stenotomum and S. gonocalyx are used to prepare special dishes by the farmers and are eaten at home. They are grown in small plots and for this reason many cultivars are sometimes not found in the markets. The most important cultivars are 'Culi', 'Pina', 'Kunurana', and 'Zapallo'.

The S. andigena cultivar 'Sicha' was mentioned by many collectors during early explorations. I looked for this cultivar in many places, but could not find it. Particularly characteristic is that it has an 8 month growing period, black skin, yellow flesh and rounded tubers. Today some tubers are found with similar characteristics and falsely called 'Sicha'. However, I made a special trip to Takesi, near Mount Murarata in Dept. La Paz, on the old road from Palca to Chulumani and there I did find many fields of 'Sicha'.

In Potosí many people talk about 'Janka papa' cultivars. Their production is mentioned for Tinkipaya, Prov. Frías, Dept. Potosí. The interesting feature of this tuber is that it must be roasted, not boiled. It has small rounded tubers with black skin. I could find no samples of this cultivar.

Cultivars of S. juzepczukii are most susceptible to virus infection than those of other species. All material of S. juzepczukii when grown at the Belen Experimental Station was seriously infected with virus.

After the introduction of 'Sani imilla', which is a selected cultivar, I observed that the most primitive forms had disappeared in many places. Although it has good yield, its quality is bad. At first, it commanded the same price as the good cultivar 'Chiar imilla'.

but last year the price was lower than that of 'Chiar imilla' because of the inferior watery tubers.

Another important species that was mentioned in many early collections is S. chaucha. Today it is difficult to find samples of it in cultivation.

In Achaccachi, Prov. Omasuyos, Dept. La Paz, before 'Sani imilla' was introduced we saw mixtures of species, but today most of the fields are cultivated with 'Sani imilla' and there are few plots with a mixture of primitive species and forms.

We conclude that in many parts of Bolivia the erosion of genetic variability is due to the introduction of selected cultivars, and many primitive forms are diminishing or have disappeared altogether."

The conclusion is inescapable that in Bolivia, even more than in Perú genetic erosion in potatoes has been progressing at an alarming rate. Indeed, it would seem that this has now arrived at a point which could be classified as an emergency, on the genetic erosion scale.

#### 9. Genetic erosion in Argentina

- a) Wild species From my experience on my 1967 expedition I do not think that genetic erosion amongst wild species has yet reached a dangerous level. No recent report has been received.
- b) Cultivated species The collections made by Hjerting and co-workers in the 50's have now been lost. Our experience in 1967 indicated that there had been a drastic diminution in the range of cultivars during the intervening period. It should be understood that I am here referring primarily to the north-western provinces where the indigenous or semi-indigenous land races were cultivated. A search should be made without delay now to salvage the last remnants of this apparently fast disappearing material.

#### 10. Genetic erosion in Chile

- a) Wild species Very few tuber-bearing wild species of Solanum occur in Chile. I have no information as to how far these



are threatened. The escaped or weedy forms of S. tuberosum may be disappearing, but again, I have no information on this.

- b) Cultivated species Genetic erosion in Chile has been particularly intense not only because the native land races succumbed to Phytophthora in the early 1950's when this disease first made its appearance there, having apparently no inherited resistance to it, but many European and North American varieties have also been introduced into that country in the last few decades. Luckily, the valuable Ochoa collection from southern Chile is being conserved in the Lima gene-bank. Further information from Ing. Ochoa and from knowledgeable Chilean potato specialists is required to see whether there is any indigenous material still existing in Chile which still needs collection and preservation.

11. Genetic erosion in Brazil, Paraguay and Uruguay

The amount of genetic erosion within wild species is entirely unknown to me.

No indigenous cultivated forms occur in these countries.

J. G. Hawkes  
January 1, 1973

# **2**

## ***Utilization of Genetic Resources***

## CONTENTS

	Page
I. Summary of Recommendations	53
II. Agenda	55
III. Introduction	59
IV. Maintenance	61
V. Documentation	61
VI. Utilization of Cultivated Diploids	64
VII. Utilization of Cultivated Tetraploids - Andigena	67
VIII. Utilization of Cultivated Tetraploids - Tuberosum	73
IX. Utilization of Cultivated Triploid and Pentaploid Potato Species	75
X. Utilization of Non-Cultivated (Wild) Species	78
XI. Conservation of Potato Germ Plasm	84
XII. Recommendations	86
APPENDIX 1	89
APPENDIX 2	133
APPENDIX 3	135

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## I. SUMMARY OF RECOMMENDATIONS

### PRIORITY I

1. Resistance to Pseudomonas solanacearum and Phytophthora infestans.
2. Adaptation to new potato growing regions.
3. Resistance to virus "Y".
4. Resistance to cyst and root-knot nematodes.
5. Selection of frost resistant S. x juzepczukii and S. curtilobum.
6. Use of meristem culture to conserve genetic resources.
7. Evaluate and maintain selected clones at all ploidy levels.
8. Improve quality and maintain high production of protein.
9. Conduct wide search for leaf roll resistance.
10. Resistance of tubers to Phytophthora infestans.

### PRIORITY II

1. Frost resistance studies in S. ajanhuiri, stenotomum and phureja.
2. Resistance to races of Synchytrium endobioticum.
3. Examination of breeding procedures.
4. Documentation of collections at other centers.
5. Utilization of further sources of resistance to P. infestans.

### PRIORITY III

1. Screening of further lines to provide a broader genetic basis of resistance to P. solanacearum.
2. Breeding at 2 x level for use in 4 x hybrids; diploid x haploid.
3. Wild species - maintain as botanical seed.
4. Need for research on physiological factors in Tuberosum x Andigena influencing tuber initiation and growth.
5. Use of botanical seed to produce crops on farm scale or as substitute for certified seed.
6. Establishment of a data handling center to collate CIP data.
7. Resistance to a spectrum of diseases and pests that are identified as the program develops.

CENTRO INTERNACIONAL DE LA PAPA

INTERNATIONAL PLANNING CONFERENCE ON THE UTILIZATION

OF GENETIC RESOURCES OF THE POTATO

II. AGENDA

Monday, April 15

9:00 Introduction of Participants

Overview of the Objectives of the Planning  
Conference and of the Center

Dr. R. L. Sawyer, Director General

9:30 I Current Status of CIP Germ Plasm Collection

II Priorities and Future Goals of the Breeding  
and Genetics Programs

Dr. P. R. Rowe, Head, Department of Breeding  
and Genetics

10:15 Coffee

10:30 Use of the Cultivated Diploids

1. Useful Characteristics

2. Strategy for Utilization

Dr. F. L. Haynes, North Carolina State University,  
U. S. A.

12:00 Lunch at La Molina

Monday afternoon

1:00 Discussion and Preliminary Recommendations on the  
Use of Cultivated Diploids



3:00 Coffee

3:15 Use of the Cultivated Triploids and Pentaploids

1. Useful characteristics

2. Strategy for Utilization

Dr. F. de la Puente, Ministerio de Agricultura,  
Peru.

3:45 - 4:30

Discussion and Preliminary Recommendations on  
the Use of Cultivated Triploids and Pentaploids

Tuesday, April 16

9:00 Use of the Cultivated Tetraploids - Andigena

1. Useful characteristics

2. Strategy for Utilization

Dr. R. L. Plaisted, Cornell University, U. S. A.

10:15 Coffee

10:30 Discussion and Preliminary Recommendations of the  
Use of Cultivated Tetraploids - Andigena

12:15 Lunch at La Molina

Tuesday Afternoon

1:00 Use of the Cultivated Tetraploids - Tuberosum

1. Useful characteristics

2. Strategy for Utilization

Dr. D. A. Young, Agriculture Canada

3:00 Coffee

3:15 - 4:30 Discussion and Preliminary Recommendations on the  
Use of Cultivated Tetraploids - Tuberosum

Wednesday, April 17

- 9:00 Use of Non-Cultivated Potato Species
1. Useful characteristics
  2. Strategy for Utilization
- Dr. J. G. Th. Hermesen - Institute of Plant Breeding,  
The Netherlands
- 10:15 Coffee
- 10:30 Discussion and Preliminary Recommendations on the  
Use of Non-Cultivated Potato Species
- 12:15 Lunch at La Molina

Wednesday Afternoon

- 1:00 General Discussion on the Conservation of Potato  
Germ Plasm for Future Use
1. Clonal Propagation
  2. Bulk Seed Conservation
  3. In vitro Techniques
- 3:15 Coffee
- 3:15 - 4:30 Continuing discussion

Thursday, April 18

- 9:00 - 4:30 Committee to Summarize Discussion and to Prepare  
Final Recommendations
- Dr. H. W. Howard  
Dr. A. O. Mendiburu  
Dr. D. A. Young  
Dr. P. R. Rowe  
Dr. O. T. Page

12:00 Other Participants  
Tour of CIP Facilities at La Molina

12:00 Lunch at La Molina

Thursday Afternoon

1:15 Other Participants  
Free for Discussion with CIP staff

Friday, April 19

9:00 Discussion of Final Recommendations and Assignment  
of Research Priorities

12:00 Lunch at La Molina

### III. INTRODUCTION

It is estimated that less than one percent of the genetic variability of Solanum has been utilized in the development of existing varieties of potatoes. Making wider use of genetic materials, and especially prospecting the germ plasma for multigenic field resistance to pests and diseases, can make enormously valuable contributions toward solving many problems in potato production.

The successful transfer of the potato from tropical to temperate latitudes, and later to tropical regions of Africa and Asia, has been due to the selection of clones adapted to the long days of a temperate growing season or the selection of day-length insensitive clones. From eons of natural selection in cool Andean habitats, the genus Solanum was also generally well adapted to grow in the relatively short frost-free season of northern regions. This wide adaptability to temperature and day-length, combined with its outstanding nutritive and yield qualities, has made the potato the fourth most widely cultivated crop of mankind.

In keeping with the philosophy of the Outreach program, the basic goal of CIP is to raise the productivity of potatoes in countries of the developing world where need and opportunity are greatest. This implies an expanded and more efficient use of the essentially untapped genetic currenty of the potato. To this end expert opinion was sought to plan how to most effectively utilize the spectrum of genetic resources available at the diploid to pentaploid levels.

The present report is based on papers summarized by opening speakers at six sessions and on a position paper prepared and circulated prior to the Planning Conference. An attempt has been made to incorporate important points raised during discussion and to summarize recommendation with suggested program priorities and expected duration of each research objective.

Three additional topics, outside of the strict interpretation of items for direct consideration, but with important aspects in relation to the utilization of genetic resources, were discussed: a) the need for more research on the physiology of tuberization; b) how CIP intends to use its Outreach program to test new potato genotypes in tropical areas; and, c) the possibilities and advantages of growing potato crops from true (botanical) seed.

In introductory remarks, Dr. P.R. Rowe, Head, Department of Breeding and Genetics outlined the two main functions of the Department:

- 1) To maintain, distribute, and coordinate the evaluation of the potato germ plasm collection of CIP, and
- 2) To utilize all available genetic resources of potatoes to synthesize segregating populations or clones that will solve problems of potato production.

To meet the challenge of creating potato clones with increased specific and general adaptation, potato breeders need to utilize the greatest possible sample of genetic diversity. For this reason, a great deal of emphasis is being given in the early stages of development on efforts to develop a large, well documented germ plasm collection. This material forms the genetic base for almost all programs in CIP and much of the future success of CIP programs will depend upon the proper utilization of these genetic resources.

The efforts of CIP to collect potatoes are based on plans developed by a planning conference in January 1973. This plan calls for the systematic collection of native cultivars in the countries of Mexico, Central and South America. The expeditions for 1974 are summarized as follows:

- |                             |  |
|-----------------------------|--|
| 1) Hawkes/van Harten/Landeo | Cusco; Puno; Bolivia; Argentina.<br>March and April. |
| 2) Hjerting/Aguilar         | Huancavelica, Junin.<br>March and April.             |
| 3) Jackson                  | Cajamarca. May.                                      |
| 4) Huamán                   | Piura. May   |
| 5) Ochoa/Egúsqüiza          | Arequipa. April.                                     |
| 6) Ochoa/Blanco             | Cusco. May   |
| 7) Ochoa                    | Pasco, Huanuco.<br>May and June.                     |

Also a short organizing visit to Guatemala and Mexico by Ochoa in July or August is planned.

#### IV. MAINTENANCE

In April, 1974, the germ plasm collection included 2650 entries of cultivars that have been given accession numbers. An additional 1900 of recent expeditions are being examined prior to the assignment of accession numbers. All primitive cultivars are maintained asexually as clones at this time. As more information about these clones is developed by CIP scientists, duplicates will be eliminated and a system for long-term propagation by botanical seed will be developed. Presently, open-pollinated seed is available for over 70% of the entries in the collection. Botanical seed of potatoes can be stored 15-20 years with current procedures.

The collection also includes over 900 entries of wild species. The maintenance of the wild species is being done in cooperation with the U.S. Potato Collection in Sturgeon Bay, Wisconsin. Duplicate samples of seed are shared by these two collection and seed increase is being done at this time in Wisconsin.

Because of the special problems that arise in the propagation of clonal material, CIP scientists are looking at tissue culture techniques as a possible aid in this work. A program to use meristem culture to free clone of pathogens has been established for use for clones that require special care and increase. In addition, CIP is looking at the use of tissue culture technique as a means of long-term storage and as a means of distributing genotypes in the future. Work on tissue culture is also the subject of special project requests for funding.

#### V. DOCUMENTATION

Data of several types are available for entries in the collection. At this time, data are kept in a manually operated system. However, an active program to determine the proper computer data processing system is underway. The Center was visited by the consulting team that is evaluating all computer needs of International Centers; their recommendations will influence the development of the computer system for CIP. Meanwhile, CIP and other major potato collections have collaborated with Dr. David Rogers of FAO on a pilot project to evaluate the effectiveness of a particular data processing system. CIP intends to collaborate on the development of a uniform documentation system. A proposal for special project funding has been developed to cover the high initial costs of implementing a computer system.

The main research projects of the Department of Breeding and Genetics are:

1) The maintenance, distribution and evaluation of potato germ plasm.

The collection of germ plasm is under a five-year plan that was developed by a workshop held in January. The introduction of clones and seed from other breeding programs is an effort to bring in material to use in countries in the temperate areas of the world where the Center may be concerned. The immediate goal is to provide full documentation for each entry in the collection. This will include place of origin, chromosome number, species name and accumulated screening data. At the earliest possible time, an inventory of the collection will be distributed so that the CIP collection can be used more readily by all researchers. Many CIP scientists are cooperating in the evaluation of the collection.

2) The development of clones with resistance to bacterial wilt.

The Phureja source of resistance to Pseudomonas solanacearum is being used to develop clones with resistance to this disease and with adaptation to other conditions in areas where bacterial wilt is a serious problem. Clones that combine resistance to wilt and late blight are being tested in several countries. As the result of plans made at a workshop on bacterial wilt, a plan for an international test of clones has been initiated. A contract project with Wisconsin (L. Sequeira) provides the basis for the orderly preliminary screening of new material for testing in the field and is developing new information on the genetics of resistance.

3) Adaptation and utilization of potato populations in breeding.

The breeding and genetics program has the responsibility of synthesizing the diverse genetic resources into potentially useful genetic combinations. The overall goal is to produce populations from which we may select clones for a wide range of environments. Contract projects are being used to increase efforts to use more of the genetic resources of the potato. A population of Andigena is being selected in New York (R. L. Plaisted). Likewise, a population of Phureja and Stenotomum is being selected in North Carolina (F. L. Haynes). Both projects are now testing clones in Peru at high and low elevations. A contract to concentrate more effort on Phureja is being developed in Peru (F. de la Puente). A project on new breeding techniques is being conducted in Wisconsin (S. J. Peloquin). A project to utilize the wild species in Mexico as new sources of late blight resistance has been initiated (J. G. Th. Hermesen). Core scientists in breeding will take the material developed by the projects and the superior genotypes that are discovered by other scientists in CIP and make crosses and select the best material for testing in Peru and other countries.

In addition to efforts to improve and utilize the germ plasm from Mexico and the Andean Region, a collection of *Tuberosum* clones from the Northern Hemisphere has been accumulated. These clones, with many years of breeding behind them, represent sources of quality and disease resistance that can be used directly or as parents in certain potato growing areas that are of interest to CIP. These clones will also be used in crosses with improved *Andigena* and *Stenotomum* clones to gain maximum hybrid vigor.

- 4) Evaluation of advanced clones, possible parental clones, and from the germ plasm collection for nutritional quality factors.
- 5) Analytical studies for protein analysis of potatoes.

These projects have been developed after the Planning Conference on potato nutrition that was held in Lima in November, 1973. It is clear that there is no general agreement on the techniques to be used for large scale testing of protein content. For this reason, a project to evaluate analytical techniques will be conducted at the same time as the work to evaluate material now in the program of CIP. First efforts will be directed towards the definition of the nutritional value of clones currently used in production and for those clones that will likely be used in the near future. Once these values are established, work on evaluating the genetic potential for improvement can begin. It is likely that clones with more extensive levels of variation can be detected in the germ plasm collection, and these will be incorporated into the breeding program.

- 6) Selection and breeding of potato clones with frost resistance.

The need for potato varieties with resistance to frost is well recognized. However, the lack of good testing procedures have limited progress in projects all over the world. For this reason, a breeding project on frost resistance was not fully activated until after a Planning Conference on frost resistance that was held in February. A freezing test that seems reliable and that has reasonable capacity is being used.

- 7) Genetics and breeding for resistance to *Heterodera rostochiensis*, in clones of *S. tuberosum* spp. *andigena*.

Breeding for resistance to the cyst nematode is limited because of the need for more information concerning sources of resistance, the variation in the nematode, and proper testing techniques for large population. At this time, preliminary work is being done as part of an M.S. thesis project. Clones thought to be resistant to two different nematode populations have been crossed and the progeny will be used to gather initial evidence on inheritance of resistance.



### 8) Control of Late Blight - Breeding for resistance

A Planning Conference in August, 1973, discussed the problems of control of late blight. The results of that Conference have been used in the development of this project. Entries in the germ plasm collection are being screened to find possible new sources of resistance. Meanwhile, clones isolated by the testing program in Mexico will serve as an immediate source of resistance in areas where these are adapted.

## VI. UTILIZATION OF THE CULTIVATED DIPLOIDS

The cultivated diploid potatoes, groups Phureja and Stenotomum, deserve much more attention than they have received. From the standpoint of organized efforts toward improvement, they have largely been neglected in favor of the tetraploids of Groups Andigena and Tuberosum. In recent years however, there has been increased interest in the possibilities of breeding potatoes at the diploid level. The research of Hougas and Peloquin and their co-workers have stimulated this interest and they have presented most of the arguments favoring breeding at the diploid level.

In considering breeding at the diploid level, emphasis is usually placed on the utilization of haploids that have been derived from the cultivated tetraploids. The cultivated diploid species have been considered primarily as sources of disease and insect resistance to be utilized as non-recurrent parents in backcross programs. Little systematic effort has been made to improve these species themselves. For example, the diploid clones currently being grown commercially in South America are largely unimproved native cultivars.

This material is a largely untapped reserve of genetic potential for greater production. New clones selected for adaptation to specific environments and for higher yields incorporated with other important traits found in these diploids would be a major contribution to potato production for both the highland and lowland tropics.

Many of the valuable traits found in the diploids have been listed below. As is usual, emphasis has been placed on resistance to pests. While these are valuable characters, we should recognize that there are other traits which may prove to be equally as valuable as many of the resistance factors.

Disease Resistance:

Virus A                      phu  
Virus X                      phu  
Virus Y                      phu, stn  
Virus Leafroll              phu, stn

Bacterial wilt (Pseudomonas solanacearum)              phu, stn

Ring rot (Corynebacterium sepedonicum)              phu

Late blight (Phytophthora infestans)              phu

Verticillium wilt (V. albo-atrum)              phu

Insect Resistance:

Flea beetle (Epitrix spp.)              phu

Aphids                      phu

Nematode Resistance:

Golden nematode (H. rostochiensis)              phu

Other cyst nematodes              phu, stn

Frost Resistance:

Found in S. ajanhuiri, S. phureja

High Dry Matter:

Very high levels found in both phu, stn

One very important character is total solids or dry matter content of tubers. In North America a plateau has been reached in breeding for increased dry matter percentage. It seems that we need new sources of genetic variation for this character as well as several others. An example of this is indicated in the work of Plaisted in which a program of recurrent selection for increased dry matter provided minimal progress in increasing dry matter percentage. In North Carolina, the average dry matter percentage for Tuberous clones is between 17.0 and 17.5 percent and the highest clones are about 19.5 percent. The range is dry matter for two families of Phureja which have been evaluated is:

Family 1	N = 18 clones	X = 19.80
	Range was 15.4 to 24.8, 7 clones over 20%	
Family 2	N = 16 clones	X = 19.05
	Range was 15.6 to 26.5, 7 clones over 20%	

In other lots of the same base population grown in Peru, the mean for 60 clones was 23.5%, and the range was 18.4% to 31.6%.

It may be argued that dry matter percentage is less important to developing countries than total yield of dry matter. This is quite true, but it is likely that the highest potential is in high yielding clones that also have a high percentage of dry matter.

Another important consideration is that of hybrid vigor. How soon we may begin to exploit the full potential of hybrids vigor depends on several factors. From the work of Plaisted; Mendiburu and Peloquin; Rowe; Haynes; and others, it appears highly probable that the full utilization of hybrid vigor will depend primarily on how much effort is expended in the identification of parental combinations with high specific combining ability from among adapted clones. There is no doubt that heterosis will be systematically exploited in the near future.

## VII. UTILIZATION OF CULTIVATED TETRAPLOIDS

### -ANDIGENA

#### 1. Useful Characteristics.

##### A. Yield of tubers.

- 1). Andean region. An irreplaceable reservoir of genetic variability exists which is subject to loss as improved varieties become available. National programs have only touched the surface of the potential for hybridization and selection. Hybrids with selected Tuberosum may prove useful.
- 2). Temperate regions. Tuberosum germ plasm is very highly selected with limited range in new potential. Andigena is the best source for an extensive infusion of new variability. Preliminary selection of the Andigena is needed to remove the portion of variability which transmits undesirable traits. Tuberosum x Andigena hybrids will result in a lower frequency of selection but a higher potential for yield. A mean progeny advantage of selected Andigena in hybrid combination with Tuberosum over Tuberosum x Tuberosum progenies tested in temperate regions has been reported to be 20% by Howard, 50% by Paxman, 13% by Glendinning, 34% by Tarn and Tai, and 15-19% by Cubillos and Plaisted. Unselected Andigena in hybrid combination with Tuberosum is too late in maturity so yields are less than intra-Tuberosum progenies. Consequently the ultimate hybrid advantage should be greater than the reported statistics. A thesis by Cubillos reveals the expression of heterosis is related to day length with maximum heterosis of 30% at 13 hours mean day length in the current stage of selection.
- 3). Other regions of world production.
  - (a) "Normal" temperature during tuberization. While the day length dependency of heterosis in Tuberosum x Andigena hybrids has yet to be confirmed, it suggests the capability of producing Andigena populations with various levels of selection for adaptation to long days and producing hybrid populations with optimum fit to specific latitudes, given the months of the growing season.

Another possibility is that in these selected Andigena populations there are clones which are neutral to day length expression. Experience has shown there are Tuberosum cultivars that are day length neutral. Selection under short day conditions of a diverse base of Tuberosum germ plasm could produce a corresponding population that would be useful in hybrid combination with the day length neutral Andigena population. If day length specificity has no intrinsic advantage, hybrids of this type would be widely useful.

- b). Warmer than "normal" climates. Within reasonable limits, variation in ability to tuberize under higher temperatures appears to exist within Andigena as well as variation for many other traits. Special programs of selection for this ability combined with resistance for the diseases encountered under these conditions should prove fruitful.

#### B. Day-Length Sensitivity.

Since Tuberosum clones are known that are insensitive to day length, it is likely the same is true within Andigena. The CIP trials being conducted at Huancayo (Peru), Toluca (Mexico) and Ithaca (U.S.A.) in 1974 are designed to indicate whether selection of Andigena populations under long day conditions will achieve this.

#### C. Maturity.

Maturity is confounded with day-length sensitivity, but is not totally determined by it. If they were needed for specific cropping patterns, Andigena with earlier maturity could be produced. It might be that the total yield per crop of the longer season varieties might be greater than of the shorter season varieties, though this is not a certainty, but it is likely that yield improvements in terms of number of days the crop occupies the land would be achieved.

#### D. Range of Adaptation.

The plasticity of Andigena for its range of adaptation has been underrated. It should also be noted that to take advantage of this value requires an extensive effort in cyclic selection. Cubillos' trials with CIP at Peru, Colombia, Mexico, and New York produced results indicating greater stability for the inter-group hybrids than either in-

tra group Andigena or Tuberosum populations, where stability is defined as deviations from the expected linear performance over locations. For those who prefer to define stability as a slope of 1.0 for variety performance plotted against location performance, the intra-Andigena population was the most stable. This interpretation for this range of environments needs caution. Even though combinations of Tuberosum parents based on tuber set and tuber size have produced no evidence of merit, these factors appear to influence the heterosis of the Tuberosum x Andigena hybrids over the range of locations evaluated in Cubillos' CIP trials.

#### E. Disease Resistance.

- (1) Late blight. Andigena has had a reputation in temperate regions for being more susceptible to Phytophthora infestans than most Tuberosum varieties. This has been a barrier to its utilization in temperate climate breeding programs. Work by Thurston, Estrada, and others in South America and by Simmonds and Thurston in temperate areas has shown that forms with high levels of field resistance can be produced. The fifth cycle of the Cornell-CIP population of Andigena was tested in Toluca, Mexico in 1973 and 37% were rated 3 or better. A sample of clones including several rated as susceptible in Mexico were grown under conditions of an artificial epiphytotic at Ithaca. All Andigena clones remained blight free while Sebago, Kennebec, Katahdin, and several other Tuberosum varieties were totally devastated.
- (2) Potato Virus Y. PVY has been a scourge of two separate Andigena populations at about the same stage of selection in Ithaca. This has not been true of Tuberosum populations grown at the same location. This has produced the impression of special susceptibility to PVY in Andigena. These same epiphytotics of PVY have revealed genetic resistance of the extreme resistance or immunity form. Attempts to infect these clones by aphids, grafting, and mechanical infection have failed. Resistance appears to be due to a single dominant allele. A few clones have shown the hypersensitive reaction of USDA 41956. The current cycle of the Cornell-CIP Andigena population has 61% of the clones with the immune reaction.
- (3) Potato Virus X. Experience such as Thurston's in Colombia indicates a relative high frequency of resistance to PVX in andi-

gena cultivars. This is borne out in the Cornell-CIP population in which 86% of the last selections are immune or extremely resistant. Except for that which may have occurred in the first cycle, there has been no selection for PVX resistance.

- (4) Leaf Roll Virus. Unknown.
- (5) Potato Virus S. Resistance in Andigena reported (see Position Paper, Appendix I).
- (6) Potato Spindle Tuber Virus. Unknown.
- (7) Bacterial Wilt or Brown rot. The Fhureja source of resistance has been combined with blight resistant clones of Andigena. The offspring have been screened for resistance to late blight and the K60 strain of brown rot. The survivors will be multiplied (un-inoculated parent clone sources) and tested at several locations for both blight and endemic brown rot. (A Cornell-CIP project).
- (8) Common scab. The Position Paper refers to reports of resistance in Andigena. Resistant clones have been identified in the Cornell-CIP population. Data are not available yet on the proportion which is resistant. These should be a valuable addition to the limited Tuberosum sources of resistance.
- (9) Wart. The Position Paper cites references to resistance in Andigena. The Cornell-CIP population will be screened by Proudfoot in Newfoundland in 1974.
- (10) Verticillium Wilt. A partial search of the Cornell-CIP population has produced clones resistant to a mixture of V. albo-atrum and V. dahliae. A more extensive examination will be made in 1974.

#### F. Insect Resistance.

- (1) Aphids. Resistance has been reported in Andigena to both Mysus persicae and Macrosiphum euphorbiae. The Cornell-CIP population has been evaluated in one location as single spaced hills; 21% had low levels of infestation. These need replicated testing in 1974.

- (2) Leaf hoppers (Empoasca). The position paper refers to published reports of resistance. The Cornell-CIP population was evaluated at Ithaca and Beltsville, Maryland in 1973. Resistant clones were identified at each location, but there was considerable inconsistency. The most promising clones will be retested in 1974.
- (3) Colorado Potato Beetle (Leptinotarsa decemlineata). No published reports of resistance in Andigena and a test of the Cornell-CIP population in Maryland in 1973 produced no indication of resistance. A more broadly based population will be re-evaluated in New York and Maryland in 1974.

#### G. Nematode Resistance.

- (1) Potato Cyst Nematode (Heterodera). The resistance found by Ellenby has been used extensively in nematode resistant breeding programs. Mayer at CIP and Ministerio de Agricultura, Peru, has evaluated a large number of Andigena at three locations in Peru and identified additional sources of resistance to an unidentified range of races. A gene, H<sub>3</sub>, has been identified which gives resistance to H. pallida.
- (2) Root Knot Nematodes (Meloidogyne spp.) As shown in the position paper, Ross and Rowe report resistance to root knot nematodes in some Andigena clones. The Cornell-CIP population was tested with four species of Meloidogyne and 13% were resistant.
- (3) Root Lesion Nematode (Pratylenchus penetrans). No reports known of resistance in Andigena; however, several Tuberosum clones with the H<sub>1</sub> gene for cyst nematode resistance also have resistance to Pratylenchus in New York tests.

#### H. Dormancy.

Scientists in South America who are familiar with Andigena, report greater dormancy in those clones than in Tuberosum clones. The Cornell-CIP population verifies that extremes in dormancy exist in Andigena beyond that encountered in Tuberosum. Four out of 300 clones, held at 70°F from harvest in September, had not sprouted by March 15. It is also true that there are some with very short dormancy. As with so many traits, the Andigena population displays a wide range in variability.



I. Vine Type.

When Andigena is grown in temperate latitudes, it has a vine growth which has been characterized by Simmonds. Both at Pentlandfield and Ithaca, selection for cropping ability has brought about a concurrent increase in proportion of the clones which have a Tuberosum-like foliage.

J. Chipping Ability.

Tubers of the Cornell-CIP population were held at 40° and 50°F and then chipped after two and 6 weeks of reconditioning at 65°F. Twenty-four percent of the clones produced chips of acceptable color under these 4 storage and reconditioning environments.

K. Flavor.

This important attribute has not been given an evaluation that can be cited and tested. Nevertheless, enough unsolicited and independent opinions have been expressed in favor of the flavor of Andigena varieties that this difference must be considered.

2. Strategy for Utilization:

A. Creation of heterogeneous populations of Andigena.

- (1) Master population at CIP designed to foster maximum panmixis among all known sources of Andigena.
- (2) One or more populations selected for adaptation to long day-temperate latitudes.
- (3) One or more populations selected for ability to tuberize under high temperatures.
- (4) Populations with special attributes. (May be separate or may be a component of one of the first 3 populations.)
  - (a) Field resistance to late blight.
  - (b) Virus resistant.
  - (c) Resistant to root knot nematode, brown rot, and late blight; probably in combination with ability to tuberize under high temperatures.

(d) Insensitive to day length

- B. Creation of a broadly based Tuberosum population selected for adaptation to low latitude or insensitive to day length.
- C. Creation of bulk hybrid populations between the Andigena and the Tuberosum populations.
- D. Distribution of bulk lots of seed of any of these populations to the Outreach programs for selection for specific adaptation or to potato breeders at National programs for the same purpose.

## VIII. UTILIZATION OF CULTIVATED TETRAPLOIDS

### TUBEROSUM

#### Useful Characteristics

Many of the useful characteristics found within Solanum tuberosum spp. tuberosum or the now wider group of Tuberosum materials which includes genetic material from S. demissum, S. andigena, S. stoloniferum, etc. are included in the position paper (Appendix 1). Additional possible characteristics of importance within Tuberosum include resistance to cracking and bruising, protein content, vitamin C content, tolerance to heat in tuber production, resistance to greening, leafspot (Macrophoma) and smut (Thecaphora solani).

In broadest terms, Tuberosum has tuber size, tuber appearance, maturity, relative daylength neutrality, and highly selected building blocks possessing a wide range of agronomic and pest resistant characteristics to offer for breeding purposes.

#### Strategy for Utilization

Variety development using Tuberosum:

1. Traditional Tuberosum breeding
2. Tuberosum-Andigena complementation
3. DH and 4x-2x applications.

### Points for discussion

1. In the position paper it is stated that: "Even when resistance has been found, it is not being used to any extent in breeding programs". This is probably an overstatement, but there is enough truth in it to strike a responsive chord with those involved in variety development. The major reason for this state of affairs is the difficulty breeders have in systematically utilizing characteristics which are important. The fact that most discard approximately 90% of populations in first selection, and the fact that perhaps only one individual in 2-500,000 is of sufficient merit for release as a variety, are monuments to this state of affairs.

Breeders do not lack genetic resources but rather lack an ability to manage in an efficient manner the resources already at hand. For this reason, a center like CIP should consider making their genetic resources available as a "package, in order to maximize the possibility of successful use of their distributed materials. The package would contain the genetic stock, a phenotypic description of that stock and a suggested strategy for utilization based on the breeding behavior of the material. At the present state of the art, the third item in the package could not be adequate or complete. It might contain a description of a procedure for evaluating the characteristic, comments on what is known of associated characteristics, and information on heritability, stability and combining ability if known. The package thus becomes a more useful entity than the genetic stock alone. However, there is still only limited knowledge of how to manipulate these genetic resources and this lack of knowledge may well be the limiting factor in their utilization.

2. Is research on potato breeding methods a valid area of investigation at CIP? CIP does not intend to breed varieties per se, but rather to rely on national programs to perform this task. The successful utilization of the genetic resources at CIP thus rests in the hands of the breeders in the national programs and their success depends on the state of the art of potato breeding methodology at the present time. This consideration could effect the priority CIP might wish to place on research on potato breeding methodology.

Stated in broadest terms the following topics are worthy of consideration for additional research:

- a) Component selection e.g. Number of stems, date of tuberization, number of tubers, tuber bulking rate, size of tubers.
- b) Indirect selection methods

- c) Selection efficiency
- d) Stability
- e) Heritability
- f) Screening procedures
- g) Radical changes in present production methods
  - 1. Direct planting of botanical seed
  - 2. Analysis of production systems to consider significant changes in plant type.

## IX. UTILIZATION OF THE CULTIVATED TRIPLOID AND PENTAPLOID POTATO SPECIES

The cultivated triploid and pentaploid potato species, according to Hawkes, include the S. x juzepczukii Buk; S. x chaucha Juz et. Buk., and S. x curtilobum Juz et Buk.

### 1. Useful characteristics

#### Triploid cultivated species

In contrast to certain wild species, the triploid cultivars are numerous and fully distributed. They constitute a high percentage in some populations due to the advantages of selection under cultivation conditions. The species listed below are considered in this group.

Solanum x juzepczukii ( $2n = 3x = 36$ ).

This species is normally cultivated at high altitudes (3,400 - 4,300 m). It is distributed from the central part of Peru up to the South of Bolivia and part of Northwest of Argentina. According to Hawkes, it is a hybridogenic species between S. acaule ( $2n = 4x = 48$ ), a wild

tetraploid species, and S. stenotomum ( $2n = 2x = 24$ ), a diploid cultivated species. It is cultivated for the production of "chuño" and "moraya".

The main characteristics of this species are:

- a) Resistance to Synchytrium endobioticum; Alternaria solani; Heterodera rostochiensis; and to frost.
- b) Hypersensitivity to virus "X", and early maturity.

This species is highly sterile. It has a photoperiodic reaction to short days and reaches yields of 15 to 20, under conditions of the highlands of Peru.

Solanum x chaucha ( $2n = 3x = 36$ ):

It is distributed from the central part of Peru to central part of Bolivia and cultivated at medium altitudes (2,500 - 3,200m). Hawkes reported some of these cultivars were found in Argentina. It is considered that this variety has been originated through natural crosses of the two cultivated species of S. tuberosum ssp. andigena and S. stenotomum.

Following are the main characteristics for this species:

- a) Immunity to Synchytrium endobioticum and virus "X"
- b) Resistance to Phytophthora infestans.
- c) Earliness.
- d) High dry matter and protein content with excellent flavor.

This species is highly sterile and accounts for approximately 1/6 of the cultivars mixed cultivations in Peru. The species has a good range of variability. Cultivars of this species, such as Huayro, Amarilla de Tarma, Huayrush, etc., are considered as the most marketable varieties in the center of Peru.

Pentaploid cultivated species

S. x curtilobum ( $2n = 5x = 60$ ).

This species is cultivated at high altitudes from the central part of Peru (Ancash) up to the South of Bolivia and between the frontier of Argentina and Bolivia. This species is indicated as a complex hybrid, developed from natural crosses between S. juzepczukii (which would produce  $2n$  gametes) and S. tuberosum ssp. andigena. This species might contain two sets of 12 chromosomes from S. acaule, one set from S. stenotomum and two sets from S. tuberosum ssp. andigena.

The main characteristics of this species are:

- a) Resistance to Phytophthora infestans, Synchytrium endobioticum, Spongospora subterranea, Alternaria solani, Fusarium sp., Pseudomonas solanacearum; and to frost.
- b) Hypersensitivity to virus "X" and virus "Y".
- c) High dry matter and protein content.
- d) Earliness.

This species has low fertility and is self-fertile. Berries are produced from free pollination and seed coming from these berries have chromosome numbers ranging from 53 to 54.

Ochoa made some crosses between this species and S. tuberosum ssp. andigena, using it as male and female, and had success in both cases. The hybrids obtained from these crosses showed (according to Ochoa) performances of 20,000 - 30,000 kg/ha. under Puno and Huancayo conditions (3,800 and 3,300-3,900 m respectively).

Zhukovskii indicates that when this species was crossed as a female parent to S. tuberosum ssp. andigena, a few seeds were produced. The hybrids were sterile, showed heterosis, and had resistance to temperatures of -1, C to -2. C and had the Andigena flavor. Bukasov reported that some hybrids of this species have good performance and are resistant to temperatures of -4. C and -5. C.

Hawkes considers that although this species is predominantly a short-day type, it has cultivars with photoperiodic reaction to long-days.

## 2. Strategy for Utilization

With the purpose of proposing strategy for utilization of triploid and pentaploid cultivated varieties, the following points are to be taken into consideration:

- a) These species are an important part of potato production in the Andean area (Peru-Bolivia). These species are popular in this area: S. chaucha for its quality, and S. juzepczukii and S. curtilobum for frost resistance and for utilization for "Chuño" and "Moraya". Puno (Peru) cultivates almost 8,000 ha. of bitter potatoes.
- b) Some useful characteristics for these species have been previously listed.
- c) Although there are fertility problems, crosses using them as male and female progenitors have been done as in the case of S. curtilobum (male) and S. chaucha and S. juzepczukii (female).
- d) The wide distribution of some triploid cultivars such as S. x chaucha cultivar Huayro for example, makes us think that they have species advantages under selection, not yet explained, and that might be profitable for improvement programs.
- e) There is a limited number of collections of these species in the CIP germ plasm bank and in some other programs. There are 70 collections of S. curtilobum, 42 of S. juzepczukii and 120 of S. chaucha.

## X. UTILIZATION OF NON-CULTIVATED (WILD) SPECIES

### Introduction

Like species in all plant families, Solanum species have built up inter-specific barriers, which prevent them from being submerged in one large Solanum - gene pool. A breeder who likes to include wild species in his breeding programme has to break the barriers between wild and cultivated species in order to make the factors accessible which are known to be stored in the wild species.

Because of the usually differences between wild and cultivated species the plant breeder has to carry out many backcrosses in order to reach the varietal level after the original interspecific cross. This is a great drawback, not only because repeated backcrossing and repeated testing are time-consuming, but also because polygenically determined characters may be lost to a great extent during the backcrossing procedure. Therefore, the recommendation of the GIP workshop in January 1973, that cultivated forms should be given first priority, is a logical one. It holds true for all crops, not only for potato. This recommendation may be extended to non-cultivated forms as follows:

When for certain characters reliance has to be placed upon non-cultivated species, those forms should be given priority, which are most closely related (or have been made most related) to the cultivated forms.

As is clear from the position paper (Appendix I) there are several characteristics for which no or too few useful genes occur in cultivated forms. In such cases the breeder has to rely mainly or even completely upon wild Solanum species.

When discussing useful characteristics, or rather, useful genes from wild species, there are two characters based on polygenes: yield and resistance to leaf roll. There are a number of independent reports on a probable contribution of genes for yield from wild species. Bukasov, Toxopeus and Ross claimed extra high yields in S. demissum derivatives, while Huijsman has obtained a number of S. vernei - derived clones with surprisingly high yielding ability. Baerecke and Ross reported a greater resistance to leafroll-infection in hybrids, derived from S. chacoense, S. acaule, Andigena and S. demissum than could be found in pure Tuberosum varieties; though resistance in the wild species themselves was not found. Both the high yields and the higher leafroll-resistance are thought to be due possibly to the complementary or other kinds of interaction between genes from the wild species and the cultivated species.

These observations are more or less incidental. However, if through systematic research they are confirmed, then the value of wild species for breeding polygenically determined characters (yield, quality characters, adaptability, horizontal resistances) would clearly be established. These observations on polygenic characters also support the hypothesis that non-race specific resistance genes from different species may be expected to differ.

Successful systematic utilization of wild species has generally been restricted to rather simply inherited characters: race-specific resistance to Phytophthora, pathotype-specific resistance to Heterodera rostochiensis and non-specific, monogenically,



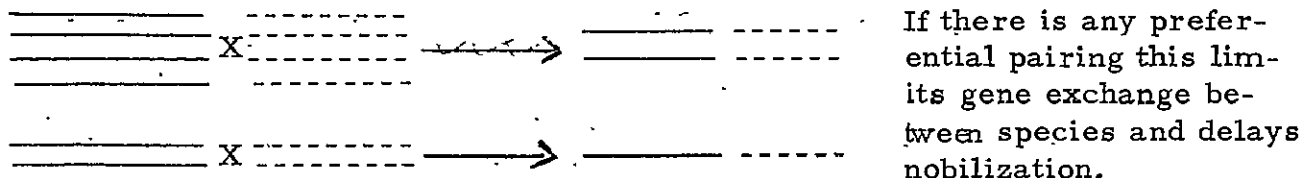
dominant immunity from the viruses X, Y and A. Typical examples of non-successful systematic attempts to utilize wild species are e. g.: frost resistance from S. acaule by Mastenbroek (due to difficult testing and complicated inheritance), resistance to Colorado beetle from S. chacoense by Torka and from S. demissum by Toxopeus (due to complicated inheritance and successful chemical control).

Genes for characters which are widespread in cultivated species, in principle need not be searched for in wild species. However, in evaluating wild species, characters which occur abundantly in cultivated species should be taken into account: the more useful and the more Tuberosum - like characters a wild species has, the lower is the number of backcrosses to Tuberosum which have to be made.

#### Fundamental statements and recommendations and strategy for utilization.

In the introduction it was pointed out that interspecific barriers, being fundamental for the existence of these species, are also unavoidable for a breeder who has to break or overcome these barriers. In addition, it was pointed out that the number of backcrosses to be made is a great drawback for the utilization of wild species. Repeated backcrosses and testings take much time furthermore it is extremely difficult to keep the level of polygenically determined characters at an acceptable level. Therefore, it was recommended to choose those wild forms which can be nobilized most quickly, in other words which are to be closely related to Tuberosum that few backcrosses are needed.

Another measure to speed up nobilization is to breed at the diploid level. This can be demonstrated with S. vernei. Usually breeders double the chromosome number of vernei to promote crossability with S. tuberosum. It is advisable to cross S. vernei with selected diploid S. tuberosum.



Without preferential pairing in the tetraploid, undesirable genes will disappear more slowly from the population, because besides ----- pairing, also ----- and ----- occur in that case.

In order to further speed up the nobilization process (and decrease

the number of backcrosses needed), it may be recommended that "pre-breeding" be carried out within wild species. Pre-breeding is a procedure comparable to the adaptation programmes which are going on in *Andigena* and in cultivated diploids.

Such pre-breeding programme should also include:

- a thorough evaluation of desired characters within the wild species before crossing with cultivated species;
- a study of the genetics of such characters within the wild species in order to avoid erratic genetic ratios due to interspecific barriers and genic genomic interactions;
- concentrating genes of the desired characters within wild species, particularly when inheritance is more or less polygenic;
- combining, within the species, different valuable characters which are scattered over different accessions of that species.

The result of a pre-breeding programme may be highly valuable clones, which are environmentally adapted and comprise concentrated genotypes for valuable characters, while also the genetics of these characters may have been clarified. Such clones are good starting material for breeding at the diploid level (or amphidiploid level). Among the species which may warrant intensive research, are: *S. chacoense*, *S. demissum*, *S. acaule*, *S. bulbocastanum* and also wild species from series *Tuberosa* like *S. vernei*.

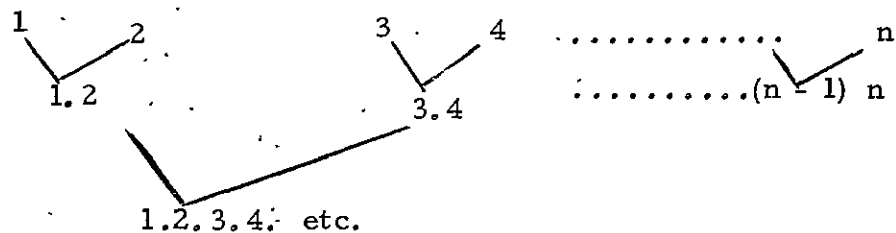
#### Principle of "resistance columns"

This method is based on the assumption, that resistance to a certain disease, in different species is based on different genes. The way of introducing a high level of resistance into cultivated forms, according to our principle of resistance columns, can generally be explained as follows:

Breed from each resistance-source resistant nobilized clones in such a way that each clone (or group of clones) derives its resistance from a different species. After having built these separate "nobilized" clones ("columns of resistance"), they should be intercrossed in order to combine the resistance genes from different species. According to this philosophy one should not be content with one source of resistance, even not with different accessions of one species. For *Pseudomonas* resistance *S. chacoense*, *Andigena* and perhaps *S. pinnatisectum* should be studied, in addition to *Phureja*.

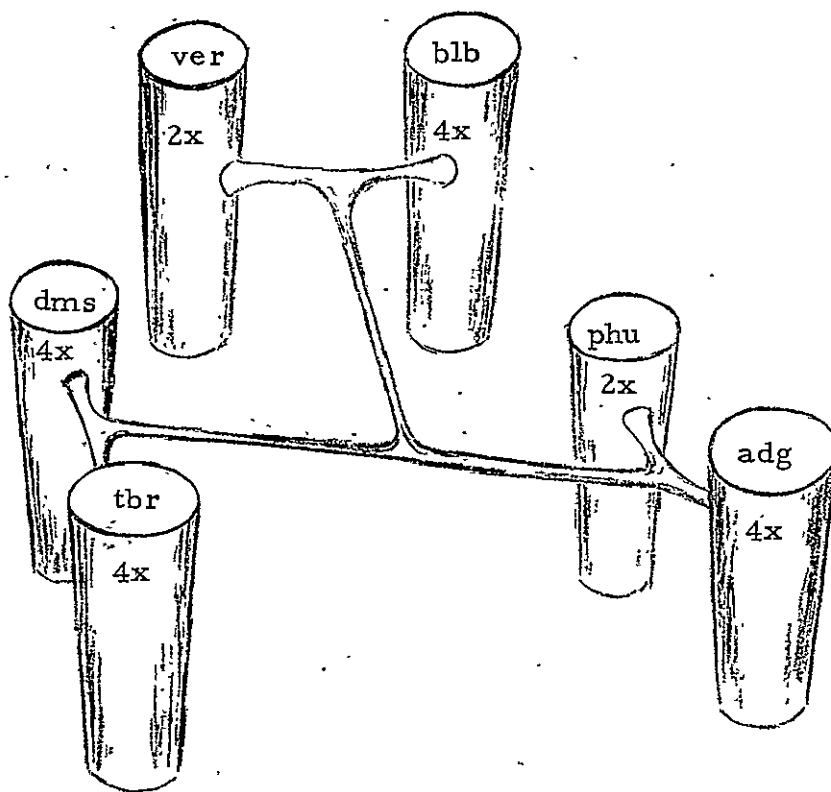
A Programme including pre-breeding, column-building and convergent breeding  
columns

	Species 1	Character x
pre-breeding	1	<ul style="list-style-type: none"> <li>Test many accessions and</li> <li>Intercross high x high, high x low, low x low</li> </ul>
Column building	2	<ul style="list-style-type: none"> <li>Determine inheritance</li> <li>Select best sib-clones</li> <li>Cross these best clones with <u>S. tuberosum</u></li> <li>Select best F plants</li> <li>Backcross</li> </ul>
	3	<ul style="list-style-type: none"> <li>Repeat procedure for backcross generation (→ column from species 1)</li> </ul>
		Build at the same time columns from other species
Combination & convergent breeding (upgrading level)	4	<ul style="list-style-type: none"> <li>Combine the columns at the "nobilized" level</li> </ul>
	5	<ul style="list-style-type: none"> <li>Test progenies, select the very best ones</li> <li>Continue convergent breeding according to the scheme:</li> </ul>



When testing has to be carried out on tubers, the pre-breeding part should be carried out on plants grown under short day conditions.

Illustration of columns for Phytophthora resistance



## XI. CONSERVATION OF POTATO GERM PLASM

### 1. Clonal propagation

Initial discussion on the conservation of germ plasm for future use was concerned with field collecting. The CIP approach was outlined, the general technique being to collect two or three tubers of each distinctive morphotype in a field. If a field had been harvested it was necessary to rely on the farmer for tuber identification, the Indian name occasionally being provided. A small group of tubers from similar plants are assigned an accession number. Every effort is made through visual observation in 15 hill plots to ascertain the purity of an accession. It was noted that flowering is general in most accessions and over 70 per cent of accessions readily set seed.

### 2. Bulk seed conservation

The possibility of bulk seed propagation as a method of germ plasm conservation was discussed at length. The obvious difficulty was in reisolating a desired recombinant. A method which tended to minimize genetic drift was outlined as follows:

Twenty numbered plants were crossed, 1 x 2; 3 x 4; 5 x 6; 7 x 8; etc., the odd numbered plants serving as female, even as males; an equal number of seeds are collected from each cross and bulked.

In another approach bulked pollen from 24 plants was used to pollinate diploids without emasculation. The usefulness of bulking on the basis of specific requirement, e. g. nematode resistance; virus resistance, was discussed.

The techniques of storing botanical seed was reviewed. Low moisture (5%) storage in impervious plastic packages, 50 seeds/pkg., at a temperature of 3-4°C permitted retention of viability normally for 10-20 years. It was noted, however, that some wild diploids could only be stored for a relatively short-time -- thus the need for testing for viability from time to time. A scheme for the production of botanical seed as a substitute for conventional method of seed certification is presented in Appendix 2.

It was suggested that CIP might maintain a catalog of tetraploid varieties and breeding stock and act as an international reference source. This would be confined to maintaining lists only.

It was noted that in selfing tetraploids there may be a loss of vigor. It was also indicated that in maintaining cultivated triploids and pentaploids that it was probably sufficient to maintain parental lines so that juzepczukii and curtilobum could be resynthesized. Chaucha may be special case in which instance seed could be maintained.

#### In vitro techniques

Although freeing selected potato clones of viruses by meristem culture technique was considered to be an acceptable technique, other tissue culture techniques were viewed less favorably. It was noted that cells in culture commonly lose their ability to differentiate after prolonged culture - even if potato cells could be induced to differentiate. The possibility of obtaining shoots from callus cultures was discussed.

## XII. RECOMMENDATIONS

(Priority I, high; II intermediate; III low).

### A. Diploid cultivated species

1. Resistance to Pseudomonas solanacearum to be combined with resistance to P. infestans.
  - (a) Pay-off in 3-5 years from work already initiated. Priority I.
  - (b) Screening of further lines to provide a broader genetic basis of resistance to P. solanacearum. Priority III (and also possibly of wild species).
2. Frost resistance emphasizing S. ajanhuiri; also use of frost resistant clones of S. stenotomum and S. phureja. Priority II, pay-off probably 5-10 years.
3. General adaptation for new potato growing regions and possible selection of day-neutral types in both diploid and tetraploid species. Priority I, pay-off possibly in 5 years and not more than in 10 years.
4. Diploid x ~~diploid~~ breeding at 2x level for use in 4x hybrids - depends on progress at places other than CIP.

### B. Cultivated tetraploids - Andigena

5. Resistance to virus Y; utilization of monogenic immunity. Resistance from S. stoloniferum to be considered as a safe-guard. Priority I, completion in under three years.
6. Resistance to races of Synchytrium endobioticum - program under-way. Priority II, results in 3-5 years.
7. Resistance to cyst nematodes. Priority I, results in 3 years (preliminary tests, 1st year; main test, 2nd year; confirmation, 3rd year).
8. Tuberosum x Andigena crosses for production of stock for breeders. Priority I, continuous, but some results in 3-5 years. Priority II, long term but results could be in 3 years. (Comparison of  $F_1$  with  $F_1 \times F_1$ ; cytoplasmic effects; etc.).

C. Cultivated tetraploids - Tuberosum

9. Documentation of collections at other centres to include parentage and information on both desirable and undesirable characters. Priority II, complete within 3 years.

D. Triploid and pentaploid cultivated potatoes

10. Selection of best bitter frost resistant varieties (S. x juzepczukii and curtilobum); elimination of virus infection; increase for use. Priority I, under 5 years.
11. S. chaucha - no recommendation until more information available.

E. Non-cultivated species

12. Resistant to cyst-nematodes (Heterodera spp.) screening with particular reference to resistance to Andean pathotypes. Priority I; screening within 3 years followed by a breeding programme.
13. Utilization of further sources of resistance to Phytophthora infestans. Priority II; 5-10 years.

F. Conservation of Genetic Resources

14. Meristem culture. Priority I; continuous service.
  - a) In vitro techniques.
  - b) Cell and tissue culture. Speculative.
15. Diploid and tetraploid cultivated species.
  - a) Preserve-open-pollinated seed of each clone initially; further maintenance by sib-crossing, etc.
  - b) Maintain clones for evaluation; keep only those clones with valuable characters. Priority I; continuous.
16. Triploids and pentaploids; Priority I; maintain for a limited period only.
17. Wild species - maintain only as botanical seeds (triploids, see 16 above).



G. General

18. High production of protein per unit area; evaluation of protein quality started. Vitamin C and other factors of nutritional importance also to be considered.
19. Resistance to leaf roll-need for a wide search to find a highly heritable type of resistance. Priority 1, (3-5 years).
20. Despite previous work, there is a need for more physiological work on factors influencing tuber initiation and growth with particular reference to *Tuberosum* x *Andigena*.
21. Basic research for 3-5 years followed by use of botanical seed to produce crops on a farm scale or as a substitute for conventional method of seed certification.
22. Resistance of tubers to *Phytophthora infestans*. Priority I (3-5 years).
23. Training programs for users of breeding material from CIP.
24. Establishment of a data handling center to collate data from CIP trials; development of a common, uniform recording terminology (See Report, Appendix 3).
25. Resistance to root-knot nematodes (*Meloidogyne* spp.); checking of suspected resistance and further screening. Priority I (3-5 years).
26. Resistance of tubers to diseases and pests.

It was recognized that many diseases and pests though locally important could not be assigned specific priority.

APPENDIX I  
POSITION PAPER ON  
THE UTILIZATION OF GENETIC RESOURCES

INTRODUCTION	<u>page</u> 90	Resistance to Other Insects	<u>page</u> 118
		Resistance to Cyst Nematodes	119
POTATO BREEDING	92	Resistance to Other Nematodes	122
The Chance of Breeding a Successful Variety	92	Discussion	124
Choice of Objectives	92	CONSERVATION OF GENETIC RESOURCES	
Choice of Parents	94		
Pollen Fertility	95	Introduction	125
Yield	95	Storage of True Seed	126
Quality	95	Pollen Storage	127
Disease and Pest Resistance	95	Induction of Flowering	127
Types of Resistance	95	Cultivated Tetraploids-	
Tests for Resistance	96	Tuberosum potatoes	127
Genetic Resources	97	Cultivated Tetraploids-	
		Andigena potatoes	128
SURVEY OF GENETIC RESOURCES	98	Cultivated Triploids and Pentaploids	128
Introduction	98	Cultivated Diploid Species	129
Frost Resistance	99	Wild Species	129
Drought Resistance	101		
Heat Tolerance	102	SCREENING FOR VALUABLE GENES	
Virus Resistance	102		
Resistance to Bacterial Diseases	109	GENERAL REFERENCES	
Resistance to Diseases caused by Fungi	111		131
Resistance to Aphids	117		

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## INTRODUCTION

Way back in 1936 Hudson wrote: "Potato breeding was", to use the picturesque words of Professor Bukasov himself (1932), "stewing in its own juice, using for the introduction of new varieties always the same old parents in innumerable combinations. A cul-de-sac had been reached, with many problems still unsolved, such as blight and virus diseases". The situation changed greatly after the first Russian potato collecting expeditions to Latin America in 1925-26, and Hawkes (1970) listed some 24 collecting expeditions made between 1925 and 1966 by non-Latin American countries and some 10 further collections made on behalf of (or with funds from) Latin American Governments or Universities. There is, therefore, in the potato gene banks, of which Hawkes (1970) listed four in non-Latin American countries and four in existence, or about to be set up, in Latin America (Mexico, Colombia, Peru and Argentina), a large number of clones of both cultivated and wild species.

While steps should still be taken to collect new material and to preserve it in gene banks before it disappears (the problem was considered recently in a CIP Workshop held at Lima in January 1973); it can be argued that the collecting of further material is not as important as testing the material already assembled for the characters wanted by potato breeders. It is all too easy for those in charge of potato collections only to keep the collections from disappearing and not to organize tests of disease and pest resistance, chemical composition and other characters. Without such tests, although the collections may be of considerable interest to taxonomists, they are of very little value to the breeder wishing to use new sources in the production of improved varieties. It would be interesting to know just how much time has been spent on maintaining collections and how little on testing the material in them. For at least one major collection, the only large-scale testing has been carried out by a few scientists not working at the station where the collection was maintained but at other places. Among the advantages of putting as much as possible of a collection into true seeds is that it minimises the amount of time spent in maintaining it and permits more resources to be given to the work of finding just what valuable genes are present (Howard, 1969).

The utilization of genetic resources, ie. the breeding of improved varieties, is a complex subject and is not confined to just listing the valuable characters found in the many known clones of potatoes. In plant breeding, just as in war, strategy (the choice of operations to be attempted) must take into account tactics (the procedure adopted for carrying out a given policy). It is, for example, pointless for a potato breeder to decide he will breed a variety resistant to frost if he has neither a frost-resistant parent which can

be used in crosses nor adequate tests for frost resistance to apply to the segregating progenies.

It is therefore necessary to consider carefully what is involved in potato breeding so that the type of genetic resources useful to the breeder can be identified. This it is proposed to do under the following headings: the chance of breeding a successful variety; choice of the objectives; choice of parents; pollen fertility; yield; quality; disease and pest resistance; types of resistance; tests for resistance; and genetic resources.

## POTATO BREEDING

### The Chance of Breeding a Successful Variety

As every practical potato breeder knows only too well, but is apparently not always appreciated by many pure scientists, the chance that any seedling will become a successful variety is very small. The position has been put very clearly by Simmonds (1969):- "In framing the objectives of a potato breeding programme the first requirement is realism. The "perfect potato" does not exist and there is good reason to think that it never will. The reasons for this statement are as follows. Consider, for example, the breeding of a maincrop ware variety. A population of seedlings of the appropriate maturity range is selected for a number of characters of economic importance; the characters are assumed to be genetically independent. Suppose we select at the 10 per cent level for the following seven characters: yield, tuber shape, cracking, cooking quality, blight resistance, leaf-roll resistance and scab resistance; at the 20 per cent level for the following seven characters: tuber size, foliage type, flesh colour, resistance to gangrene, resistance to skin-spot, resistance to dry rot and resistance to black leg; and at the 50 per cent level for three characters: skin colour, resistance to wart and resistance to virus Y. The severest selection here is at 10 per cent, which is quite weak; and yet a clone which would satisfy all these 17 criteria would emerge only once in about 10 million seedlings. If, by contrast, all 17 characters were selected very weakly at the 50 per cent level, then one selection would emerge in about 150,000 seedlings (which represents about four years' production at Pentlandfield). The conclusion is clear; one must either select for fewer characters or select less intensively. Negative correlations between characters would make effective selection even more difficult".

### Choice of Objectives

Because the chances of breeding successful varieties is so small, it is obvious that the choice of breeding objectives is very important and must be considered very carefully. It may be necessary to take into account several factors in deciding which are the most important problems to be tackled and which are of relatively minor importance.

There may be one or two deficiencies in the varieties being grown which are of outstanding importance. Such deficiencies will obviously vary from country to country. In those countries where potatoes are widely grown and where the agricultural extension services are well organized, it may be easy to obtain the necessary information. This, however, is not always so and it may need surveys and field experiments to determine the most serious problems which the breeder should tackle. For example, late blight (Phytoph-

thora infestans) was for many years considered to be a very serious disease in England, but the survey work of Cox and Large (1960) - also quoted by Howard (1963) and Howard, Johnson, Russell and Wolfe (1970) - showed that years of heavy blight attack were years of high yield, not low. Because the yield of potatoes in England depends largely on there being a sufficient rainfall, the heavy attack of blight in wet years was only removing that part of the crop surplus to a static requirement - this would not, of course, apply to all areas but only to the majority in England where blight is not severe until much tuber growth has taken place. Immunity (very high resistance) to wart disease (Synchytrium endiobioticum) is possibly another example in western Europe at the present time - although this is demanded of all new varieties, two old susceptible varieties, Bintje in the Netherlands and King Edward in England, are very widely grown without wart being found on them. Conversely survey work in England on reductions in yield from Heterodera infestations (Brown, 1969) has emphasized the importance of breeding for resistance to this pest by showing that populations of the nematode not high enough to produce obvious symptoms on the tops were reducing yields considerably.

Although the use of resistant varieties is always the cheapest method of controlling a disease or pest, there may be circumstances where chemical control or other means is satisfactory and it is not necessary to give the potato breeder a difficult problem to solve. There may, for example, be no need to breed varieties resistant to virus diseases if good "seed" districts are available. Similarly the use of stocks raised from stem cuttings may obviate the need for varieties resistant to blackleg (Erwinia carotovora var. atroseptica), to skin spot (Oospora pustulans) and some other tuber diseases (Hardie, 1970; Calvert, 1973). Protein content is another possible example - it may be easier to produce high yielding varieties of normal protein content so that legumes could be grown on the area made vacant rather than attempt to breed potatoes with high protein content.

On the other hand, it may be that in the future there will be more use of integrated control, ie. using both a resistant variety and some other method of control simultaneously. This is being used in the Netherlands for combating cyst nematodes (Nollen and Mulder, 1969), treatment with a nematicide being combined with the use of resistant varieties. Similarly good control of late blight can be obtained in England by fungicide treatment of the foliage plus varieties which have a high resistance of tubers to infection (Howard, Johnson, Russell and Wolfe, 1970). Control of virus diseases can involve growing in a good "seed" district where there are no, or few, aphids to spread some viruses plus either resistant varieties or serological testing to control the viruses spread by leaf contact.

### Choice of Parents

Having decided what criteria are wanted in new varieties, the breeder has to choose the parents which between them have the desired qualities. Normally, these will include varieties of cultivated tetraploid potatoes, either Tuberousum potatoes (ie. Solanum tuberosum spp. tuberosum) in temperate zones, or Andigena potatoes (ie. S. tuberosum spp. andigena) in tropical latitudes with day lengths not differing greatly from 12 hours.

If the varieties used in a breeding programme can be confined to the cultivated tetraploid potatoes, then most progeny will have tubers of at least fair quality and there will not be a high proportion of seedlings with bad quality as so often occurs when a wild species has been included as a parent.

If not, all the desired characters can be obtained in the tetraploid group of cultivated potatoes, then it would seem to be best next to see whether the desired character or characters can be found in the diploid cultivated potatoes rather than in wild species (Howard, 1970, 1973). There is no difficulty in crossing cultivated diploids and tetraploids, and the result of such crosses is often tetraploid, not triploid, offspring (Marks, 1966). Alternatively, breeding in the future might be at the diploid level using the cultivated diploids and dihaploids of S. tuberosum.

If neither the tetraploid nor diploid cultivated potatoes contain all the characters wanted, then resource may have to be made to wild species. Many wild species can be crossed easily with cultivated potatoes, but there are, of course, certain groups of wild potatoes and some species which have not so far been crossed with cultivated varieties. Breeding from wild species must take longer than breeding from cultivated varieties only because a series of backcrosses to cultivates has to be carried out. In addition, as has already been pointed out, it may be difficult to obtain adequate cooking quality. On the other hand, this is not impossible and a number of widely grown varieties of good quality have been obtained from wild species, eg. Kennebec in the USA, Maris Peer and Pentland Dell in the UK.

Breeding from wild species does not usually involve sterility problems in the offspring which cannot be overcome. Also, with the potato being reproduced commercially by vegetative reproduction, there are no problems as in most other crops of having to obtain progeny which breed true to type from sexual reproduction.

### Pollen Fertility

The lack of pollen-fertility in many Tuberosum varieties can be a major difficulty in the use of parents with the desired characters. On the whole, although pollen sterility may arise from the interaction of the cytoplasm of one species with the nuclear genes of another (Grun, 1970a and b), it is often possible when using Andigena varieties, diploid cultivated potatoes, and wild species to obtain a high proportion of pollen-fertile offspring to use in subsequent stages of breeding programmes.

### Yield

No new variety has any chance of success unless it yields as high as that of existing varieties. This again makes breeding from wild varieties a slower process usually than breeding from cultivated varieties only, but, as with quality, several varieties bred from wild species are very high yielding.

### Quality

It is not proposed to discuss quality as there was a Workshop on Potato Quality held at CIP, Lima, in November 1973. It should also be noted that Quality will be the theme of the 1975 Triennial Conference of the European Association for Potato Research.

### Disease and Pest Resistance

Most potato breeding has for many years been concerned with breeding for resistance to diseases and pests. Much has been achieved but there is still much to do. As has been emphasized previously (the Choice of Objectives), the first necessity is to identify the important diseases and pests and to determine whether the use of resistant varieties is the best method of control. It then remains to decide what type of resistance is required, whether there are sources of resistance, and whether there are adequate tests of resistance suitable for use by breeders.

It should also be noted that the breeding of disease and pest-resistant varieties may be the easiest way of increasing yields.

### Types of Resistance

In breeding for resistance to many diseases and pests of all crops, there appears to be general agreement that in the future much greater attention should be given to non-specific resistance because it is expected to be nearly



always more durable than race-specific resistance. These two types of resistance are also distinguished as "horizontal" and "vertical" resistance - they were defined by van der Plank (1963; page 174) as follows: "When a variety is resistant to some races of a pathogen, we shall call the resistance vertical or perpendicular. When the resistance is evenly spread against all races, we shall call it horizontal or lateral".

Race-specific resistance is often due to a hypersensitive reaction of the host and is often controlled by major genes, eg. the R genes for resistance to Phytophthora infestans.

Non-race-specific resistance is also sometimes called field-resistance. It is often due to several causes and may be controlled by polygenes. Although it may be more durable than race-specific resistance, it may be a much more difficult type of resistance for the breeder to use, particularly if it is derived from a wild species as the polygenes will tend to be lost in a back-crossing programme.

Tolerance, ie. little reduction in yield when infected, is also considered as a type of resistance. There are, however, on the whole few investigations of tolerance.

Obviously in breeding for race-specific resistance, it is necessary to know how many pathotypes (physiologic races) of the pathogen exist and how quickly new pathotypes can arise and spread. There is in addition always the possibility that some countries do not have as many pathotypes as others, particularly as many diseases and pests are confined to potatoes and not endemic outside S. America. It may also, therefore, be more difficult to breed for resistance in S. America, especially if it is of the race-specific type.

### Tests for Resistance

Adequate tests are obviously necessary in searching for disease- and pest-resistance, but they are just as necessary for the breeder in testing his segregating progenies. It is particularly important both in the large-scale screening of material and in the selection of progenies that the tests should be relatively simple to perform so that the rapid testing of many samples can be done.

It is also important to ensure that the material tested should be the correct type. This applies particularly to testing under the 16-18 days found in temperate regions of wild species, cultivated diploids, and Andigena potatoes adapted for tuber-formation under short days. Misleading results can arise from such tests.

### Genetic Resources

This rather long preamble on Potato Breeding has been given before the detailed Survey of Genetic Resources because it is suggested that the latter should be realistic - there are all too many general reviews on the uses of Latin American cultivated and wild potato species and all too few which consider the problems critically and in detail.

It is suggested that particular attention should be given in considering Genetic Resources to:

- (a) The importance of the disease, pests or other hazards - this will vary from country to country.
- (b) Possible sources of resistance - Tuberosum, Andigena, cultivated diploids, and wild species.
- (c) The type of resistance - race-specific, non-race-specific, tolerance.
- (d) The inheritance of resistance.
- and (e) Adequate but quick tests of resistance.

## SURVEY OF GENETIC RESOURCES

### Introduction

Many diseases and pests can attack potatoes and those considered below are only a selection and, moreover, a selection influenced by the author having no experience of potato growing except in western Europe. It might be useful for CIP to compile a world list of potato diseases and pests and to include in this list the importance of each disease or pest, and, where possible, sources of resistance.

In compiling the data use has been made of "Plant Breeding Abstracts" from 1960 to 1973 inclusive. It was necessary to make a stringent selection of references. More useful information could no doubt have been obtained had there been time to refer also to other abstracting journals such as "Review of Plant Pathology" (formerly "Review of Applied Mycology"), "Review of Applied Entomology: Series A, Agricultural and "Helminthological Abstracts".

Many references prior to 1960 can be found in the two publications:

Swaminathan, M. S. and Howard, H. W. (1953). "The cytology and genetics of the potato (*Solanum tuberosum*) and related species". Bibliographia Genetica 16, 1-192.

Howard, H. W. (1960). "Potato cytology and genetics, 1952-59". Bibliographia Genetica 19, 87-216.

For Latin-American countries, Montaldo (1964, 1967, 1969) has compiled the very useful lists of publications: Bibliografía Latinoamericana sobre Papas.

Bulletin 533 of the College of Agricultural and Life Sciences, the University of Wisconsin, Madison, Wisconsin - "Inventory of tuber-bearing *Solanum* species" by Ross, R. W. and Rowe, P. R. is very useful in listing sources of resistance in IR-1 Potato Collection. It gives, where known, resistance to:

1. viruses A, X, Y and Leaf Roll with occasional notes on others (S, Spindle Tuber, F, B, C, G, M and Tobacco Mosaic).
2. late blight (*Phytophthora infestans*) and Verticillium wilt with occasional notes on others (early blight, Fusarium dry rot, wart, Fusarium wilt and common scab).

3. Ring rot and Bacterial Wilt.
4. Golden nematode with occasional notes on others (Root Knot, Horse-nettle Cyst and Osborne's Cyst).
5. Peach aphid, potato aphid, leaf hopper with occasional notes on others (flea beetle and Colorado beetle).

### Frost Resistance

Potato varieties with foliage resistant to or tolerant of frost could be very useful in certain areas. Late spring frosts can have devastating effects on early crops and planting in many areas is normally delayed until frosts are not expected. Frosts may also terminate the growth of late-maturing varieties before tuber growth has ceased.

1. BUDYKINA, N. P., DROZDOV, S. N. and SINEL'NIKOVA, V. N. (1971). (Comparative frost resistance of wild potato species). Trudy po Prikladnoi Botanike, Genetike i Selekti 46, 63-69.
2. DEARBORN, C. H. (1969). Alaska Frostless, and inherently frost resistant potato variety. American Potato Journal 46, 1-4.
3. FIRBAS, H. (1962). Zusammenhänge zwischen Trockenheit und Frostresistenz. Zeitschrift für Pflanzenzüchtung, 48, 29-35.
4. FIRBAS, H. (1962). Reaktion von Wildkartoffelarten unterschiedlicher Frostresistenz auf Kalteeinflüsse verschiedener Stärke und Dauer. Zeitschrift für Pflanzenzüchtung, 48, 101-105.
5. FIRBAS, H. and ROSS, H. (1961). Züchtung auf Frostresistenz bei der Kartoffel. I. Über die Frostresistenz des Laubes von Wildarten und Primitivformen der Kartoffel und ihre Beziehung zur Höhenlage des Artareals. Zeitschrift für Pflanzenzüchtung 45, 259-299.
6. FIRBAS, H. and ROSS, H. (1962). Züchtung auf Frostresistenz bei der Kartoffel. II. Über die Frostresistenz der Knolle und ihre Beziehung zur Frostresistenz des Laubes. Zeitschrift für Pflanzenzüchtung 47 51-56.
7. HAWKES, J. G. (1962). The origin of Solanum juzepczukii Buk. and S. curtilobum Juz. et Buk. Zeitschrift für Pflanzenzüchtung 47, 1-14.

8. RICHARDSON, D. G. and ESTRADA RAMOS, N. (1971). Evaluation of frost resistant tuber-bearing Solanum hybrids. American Potato Journal 48, 339-343.
9. ROSS, R. W. and ROWE, P. R. (1965). Frost resistance among the Solanum species in the IR-1 potato collection. American Potato Journal 42, 177-185.
10. ROSS, R. W. and ROWE, P. R. (1969). Utilizing the frost resistance of diploid Solanum species. American Potato Journal 46, 5-13.
11. SUKUMARAN, N. P. and WEISER, C. J. (1970). A possible standard freezing test for evaluating tolerance in potato varieties. American Potato Journal 47, 360 (Abstract).

#### Sources of resistance

- (a) Tuberosum potatoes - none
- (b) Andigena potatoes - none? Ref. 5 suggests some.
- (c) Cultivated triploids and pentaploids - resistance in S. juzepczukii and to a lesser extent in S. curtilobum - to be expected because these are hybrid "species" with S. acaule (see below) as a parent (Ref. 7).
- (d) Cultivated diploids, Ref. 5 suggests some in some varieties of S. ajanhuiri and S. goniocalyx.
- (e) Wild species - all authors agree that S. acaule has the highest resistance (Ref. 1, 4 & 5). But resistance is also found in other species (Refs. 9 & 10) including many diploids such as S. boliviense, S. canasense, S. chromatophilum, S. commersonii, S. megistacrolobum, S. multidissectum, S. raphanifolium, S. sanctae-rosae, S. sogarandinum, S. spigazinii and S. vernei.

#### Tests:

Not easy to decide - short severe frost more damage than prolonged mild frost (Ref. 4); hardening of plants before test (Ref. 1); plants receiving only sufficient water to prevent wilting were more resistant than plants grown under damp conditions (Ref. 3); possible test based on detached leaflets. (Ref. 11); tuber resistance often associated with foliage-resistance (Ref. 6).

Inheritance - presumably due to polygenes and hybrids inherit at least some resistance (e.g. Ref. 8) and a variety with some frost resistance has been bred (Ref. 2).

#### Further work:

Probably not easy to use the S. acaule source of resistance and might be better to investigate diploid species sources. It would be interesting to have more information on how resistant are forms of the two cultivated species, S. ajanhuiri and S. goniocalyx.

It also needs to be decided on how frost resistance is inherited and the degree of resistance which it is likely can be achieved. Would this degree be adequate?

It may be worth noting that very little progress has been made in utilizing sources of frost resistance, e.g. Ref. 1, published in 1971, is still comparing wild species and confirming that S. acaule has the highest resistance without previous hardening.

Are there any areas where it would be possible to rely on having a frost every year, or nearly so, in order that laboratory tests could be compared with results in the field?

#### Drought Resistance

As is well known to every practical potato breeder who raises his stocks in a good seed district of high rainfall and tests his stocks in an area of relatively low rainfall, big differences occur in performance of clones in the two districts. Many clones which perform well in the high rainfall area are too short and low yielding in the drier area. Whether it would be possible to produce varieties with high tolerance of drought is, however, a much more difficult problem.

Only one recent reference on drought resistant was found:

RANA MUHMAMAD SALEEM and MUHAMMAD-SHAFI (1966). Drought resistance studies in different potato varieties and interspecific hybrids in West Pakistan. West Pakistan Journal of Agricultural Research 4, 99-120.

The authors claim that certain Tuberosum x Andigena and Tuberosum x S. commersonii hybrids showed better drought resistance than Tuberosum varieties. Drought resistance was also recorded by Ross and Rowe (IR-1

Potato Collection, see page 7) in lines of Tuberosum from Chile, in S. sparsipilum and S. gandarillaii. It presumably may exist in many wild species.

#### Further work:

The physiology of drought resistance in crop plants is a difficult subject - it is obviously no advantage to have a genotype which resists drought by closing its stomata to combat water loss and at the same time cannot get the carbon dioxide necessary to make dry matter. Active work is being done with other crops and it may be advisable to wait the results of this physiological work before investigating the problem with potatoes.

#### Heat Tolerance

Heat tolerance, as opposed to drought resistance, has been considered in the following three publications:

KHANNA, M. L. (1966), Breeding potato varieties tolerant to higher thermoperiods. Current Science 35, 143-144.

OCHOA, C. (1965). Antarqui, nueva variedad de papa precoz, tolerante al calor y auto-estéril. Anales Científicos (Lima) 3, 385-388.

SALEEM, R. M. and SHAFI, M. (1965). Heat resistance studies in different potato varieties and interspecific hybrids in West Pakistan. West Pakistan Journal of Agricultural Research 3, 85-102.

It appears that Andigena varieties may have more heat tolerance than Tuberosum varieties. The variety Antarqui, bred from a Andigenum x Tuberosum double-cross was claimed to be tolerant of 18-24°C.

#### Further work:

It would appear that breeders interested in heat tolerance should investigate Andigena crosses.

#### Virus Resistance

The following types of virus resistance in potatoes are recognized:

tolerance, infection resistance, hypersensitivity which often gives 'field immunity', and extreme resistance or immunity. To these four types of resistance can be added a fifth, vector resistance. This is resistance to the aphid or other vector and not to the virus itself.

Tolerance to virus infection is often considered to be of small importance in potatoes, or even undesirable because, with roguing not easy, stocks of tolerant varieties may act as a reservoir of the virus. Under certain conditions, however, tolerance may be valuable. For example, in the Isle of Jersey (Channel Isles), it is only the tolerance of the main variety, Jersey Royal (International Kidney), to virus Y that allows this variety to be grown year after year in an environment where aphid populations are high and where "seed" is not imported from a good seed growing district.

There are many viruses (including viroids and mycoplasmas) which can infect potatoes and not all of them occur in every country where potatoes are grown. There is evidence for several more potato viruses, or more strains of viruses, occurring in South America than in Europe, etc. - for example, see the following references:

DIAZ MORENO, J. (1966). Incidencia del virus del amarillamiento de venas en papa en el Ecuador y su transmisión a través de los tubérculos. Turrialba 16, 15-24.

MONASTERIOS DE LA TORRE, T. (1966). Presence of viruses in Bolivian potatoes. Turrialba 16, 257-260.

McKEE, R. K. Virus infection in South American potatoes. European Potato Journal 7, 145-151.

Obviously great care should be taken with Potato Collections not to introduce new viruses, or a new strain of a virus already present, into the potato stocks of any country. Potato Collections do tend to be heavily infected with some viruses - see, for example,

ZADINA, M. (1970). (The distribution of virus M in a world collection of potatoes). Rostlinna Vyroba 16, 721-726 - of 630 stocks examined, only 146 were not infected with virus M, an uncommon virus in many parts of Europe. That a newly introduced strain of a virus can have serious effects was shown by the tobacco veinal necrosis strain of virus Y, see:

BRÜCHER, H. (1969). Observations on origin and expansion of Y<sup>N</sup> virus in South America. Angewandte Botanik 43, 241-249.



To a large extent the amount of virus in a Potato Collection can be reduced by putting it into true seed as most viruses are not transmitted through true seed - there is, however, see below (Potato Spindle Tuber Virus) at least one important exception to this rule.

There is an extensive literature on potato viruses and it has been difficult to decide how to treat sources of resistance to them. As already emphasized for scientific work on potatoes generally, until recently most work on potato viruses has been done in the USA, Europe and other developed areas. This must have affected greatly ideas on the importance of the various viruses.

The way in which a virus is spread may be important in assessing its control and also its economic importance. The latter may also depend upon whether good "seed" districts exist and possibly also on whether reliable and quick serological tests are available to assist in the inspection and roguing of seed stocks.

Many sources of virus resistance are listed by Ross and Rowe (IR-1 Potato Collection, see page 7). A recent publication:

COCKERHAM, G. (1970). Genetical studies on resistance to potato viruses X and Y. Heredity 25, 309-348, is very useful in showing how widespread both in cultivated and wild species are genes for resistance to virus X and to virus Y (including virus A) and that some of the genes in different species appear to be allelomorphic. German work on resistance to virus diseases has been extensive; summaries of the work can be found in:

ROSS, H. (1961). Die Züchtung auf Virusresistenz bei Pflanzen. Berichten der Deutschen Botanischen Gesellschaft 74, 23-25.

ROSS, H. (1966). The use of wild Solanum species in German potato breeding of the past and today. American Potato Journal 43, 63-80.

Potato Spindle Tuber Virus - this is a viroid (see DIENER, T. O. (1972). Viroids. Advances in Virus Research 17, 259-313) and is spread by many means, including leaf contact. It is also transmitted through true seed and possibly to some extent through pollen. Infected plants cannot be recognized by serological tests. Test plants for mild strains are not satisfactory (but see below).

Sources of resistance:

MANZER, F. E., AKELEY, R. V. and MERRIAM, D. (1964). Resistance in Solanum tuberosum to mechanical inoculation with the potato spindle-tuber virus. American Potato Journal, 41, 411-416.

BAGNALL, R. H. (1972). Resistance to potato viruses M, S, X and spindle tuber virus in tuber-bearing Solanum species. American Potato Journal 49, 342-348.

The first reference claimed resistance very occasionally in Tuberosum progenies, the second in some forms of the wild species, S. guerroense, S. hjertingii and S. multidissectum (see also Ross & Rowe, 1965, ref. on page 7).

Importance: mild strains probably depress yield by 10%; infection by severe strains produces much more damage.

Future work: not an easy virus to work with, especially as it is dangerous to have it near healthy potato stocks which can easily become infected. A good tester plant for mild strains would be useful for any work and also for testing accessions in collection. This may have been found in Scopolia sinensis - see:

SINGH, R. P. (1973). Experimental host range of the potato spindle tuber 'virus'. American Potato Journal 50, 111-123.

Potato Virus S - ubiquitous; spread by leaf contact; infected plants can be recognized by serological tests; reduction in yield not more than 5-10%.

Sources of Resistance:

Tuberosum potatoes - VULIC, M. and HUNNIUS, W. (1967). Zur "Immunität" der Sorte Saco gegenüber dem S-virus der Kartoffel. Züchter 37, 243-245 - tests show the USA variety Saco to be highly resistant but not immune to S virus - also found by others.

Andigena potatoes - BAERECKE, M.-L. (1967). Überempfindlichkeit gegen das S-virus der Kartoffel in einen bolivianischen andigena - Klon. Züchter 37, 281-286. - gene Ns for hypersensitive type of resistance in the Andigena potato, clone PI 1258907.

Wild species - BAGNALL, R. H. (1972). Resistance to potato viruses. M, S, X and spindle tuber virus in tuber bearing Solanum species. American Potato Journal 49, 342-348. - resistance was found in S. gigantophyllum (may also be resistant to virus M); S. boergii, S. caldasii and S. emmae.

Future work: If resistance to virus S is required, then the Andigena source (PI 1258907) may be adequate, particularly as it appears to be due to a single dominant gene. A search for similar genes in other Andigenas might be worthwhile. On the other hand, virus S is not one of the most serious potato viruses and control can be achieved by serological testing of plants in seed stocks.

Potato Virus X - ubiquitous; spread by leaf contact; exists in many strains. Reduction in yield from mild strains may be not more than 5%, but combined with another virus such as A, effect on yield may be large. Infected plants can be recognized by serological tests.

Sources of resistance: they have been summarized by Cockerham (Ref. on page 12).

Tuberosum potatoes: Nx and Nb genes give hypersensitive reactions resulting in field immunity to certain strains only.

Andigena potatoes: Rx in USDA 41956 (bred from an Andigena) and a similar gene in CPC 1673 (the main source of resistance used in breeding varieties resistant to pathotype of Heterodera rostochiensis) gives extreme resistance to all strains. This gene is in many new Dutch varieties. Another gene in Andigena controls hypersensitive reactions to all strains of virus X.

Wild species: genes controlling extreme resistance to all strains of virus X have been found in S. chacoense and S. acaule, also possibly in S. microdon-tum.

Future work: The CPC 1673, USDA 41956 and other Andigena sources should be sufficient for breeders. As with virus S, resistance to virus X is not at present given a high priority in many breeding programmes - possibly because of control by serological testing and only minor effects on yields. On the other hand, varieties resistant to virus X are much easier to handle in producing certified seed than susceptible varieties.

Potato Virus Y (including Potato Virus A) - a non-persistent virus spread by aphids (therefore control by systemic insecticides not so efficient as for leaf-roll); exists in many strains; e.g. tobacco vein necrosis; potato virus A and C; and other more typical Y types may cause large reductions in yield.

Sources of Resistance: for genes giving extreme resistance see Cockerham (1970) - reference on page 12. Resistance to infection of a fairly high degree can be found and bred for, see for example:

LANA, E. P. and BENSON, A. P. (1967). Controlled testing and breeding for field resistance to potato virus Y. American Potato Journal 44, 128-136. Tolerance of a high order to virus Y also occurs in some varieties.

Tuberosum potatoes - gene Na is present in many varieties and give field-immunity to virus A but not to the typical strains of virus Y (in some varieties it is closely linked to Nx). Infection resistance can also be found, e.g. in the British variety Pentland Crown, to typical virus Y strains. (There is also a gene Nc giving field immunity to virus C, an aberrant strain of Y, but this is not of any practical importance).

Andigena potatoes - Na and Nc genes occur but no genes giving extreme resistance to typical strains of Y.

Cultivated diploids - probably as Tuberosum and Andigena.

Wild species - very valuable as sources of extreme resistance to all strains of virus Y. They occur in:

S. chacoense  
S. microdontum  
S. demissum  
S. hougasii  
and S. stoloniferum

At least, the S. stoloniferum genes have been transferred to Tuberosum clones of about commercial standard (Dr. T.M.W. Davidson at the Scottish Plant Breeding Station) and there must be further, probably unpublished, work on the utilization of these sources of resistance.

Future work: Obviously, should be on utilizing the sources of resistance in the above wild species. It would be advisable to check the resistance of material bred from such sources against a wide range of Y isolates and also to make sure that it functions efficiently in the field.

Leaf Roll Virus - transmitted by aphids; a persistent virus and some control can be achieved by systemic insecticides. Nearly always causes severe reductions in yield, but some varieties fairly tolerant. Infection resistance

also occurs.

#### References:

BAEREOKE, M-L. (1961). Erfahrungen mit einjährigen Kartoffel-abauversuchen unter starken Blattroll-Infektionsbedingungen. Zeitschrift für Pflanzenzüchtung 45, 225-253.

HAMMAN, U., GALL, H. and MOLLE, K-H. (1968). Erfahrungen bei der Prüfung von Kartoffelzuchtmateriel auf Blattrollvirusresistenz in Laboratorium. Theoretical and Applied Genetics 38, 85-89.

MacKINNON, J. P. (1970). Comparative levels of leaf-roll virus resistance in potato varieties and seedlings. American Potato Journal 47, 444-446.

SIKKA, L. C. and MUNRO, J. (1968). Resistance to the potato leaf roll virus in certain *Solanum tuberosum* seedlings. Indian Phytopathology 21, 161-170.

All the above references refer to infection resistance in *Tuberosum* material. The only reference known to me on other possible types of resistance is in Ross (1966- full ref. on page 12) in which it is said that Dr. Baerecke found in *S. raphanifolium* "a strong intolerance reaction to the leaf-roll virus".

Ross and Rowe, (1969-IR1 Potato Collection) lists resistance as occurring in several accessions, mostly wild species. The data are presumably from unpublished reports.

Further work: Resistance to leaf-roll appears to be a very difficult problem and a good source of resistance does not seem to be available. Using the present known type of resistance to infection, which appears to be controlled by polygenes, makes it very difficult to find clones with the many other qualities needed in a commercial variety. Resistance to leaf-roll will presumably become more important when there are many varieties resistant to virus Y. A possible solution is to breed varieties resistant to the aphid vectors.

Other viruses - in addition to the viruses already mentioned there are several others which are of local importance. They include:

Potato Mop-top virus (CALVERT, E.L. (1968). The reaction of potato varieties to potato-mop-top virus. Records of Agricultural Research in Northern Ireland, 17, 31-40). An interesting virus in that the vector is the

fungus Spongospora subterranea; was not noticed to be important until viruses A, Y and Leaf-Roll had been all but eliminated in seed stocks.

Tobacco Rattle Virus - transmitted by migratory nematodes; important in certain areas of western Europe as the main cause of spraing; References: RICHARDSON, D. E. (1970). The resistance of some potato varieties to spraing caused by tobacco rattle virus. Journal of the National Institute of Agricultural Botany 12, 112-118; SEPPANEN, E. (1972). The reaction to some potato varieties to spraing caused by tobacco rattle virus. Journal of the Scientific Agricultural Society of Finland, 44, 76-82. Apparently, immune Tuberosum varieties are known, e. g. Bintje and Record; no published results but immunity may be due to a single dominant gene.

#### Resistance to Bacterial Diseases

There are three important bacterial diseases of potatoes: blackleg (Erwinia phytophthora Appel or Erwinia carotovora (Jones) Bergey et al. var atroseptica (van Hall) Dye), ring rot (Corynebacterium sepe-donicum (Spiek. & Kotth.) Skapt. & Burkh.) and brown rot or bacterial wilt (Pseudonomas solanacearum E. F. Smith).

Blackleg - control of blackleg should be possible in the future by the stem-cutting technique of producing seed stocks. Although there was work in the 1950s on screening wild species for resistance, this has now apparently ceased. Reaction to blackleg is not given in Ross and Rowe (1969-IR-1 Potato Collection). Two recent references have been found:

DOBIAS, K. (1970). (The resistance of varieties of a world collection of potatoes against blackleg (E. carotovora (Jones) Holland). Rostlina Vyroba, 16, 687-692.

KOROMYSLOVA, M. I. (1972); (Initial material for breeding potatoes for resistance to blackleg). Byulleten' Vsesoyuznogo Ordena Lenina Institute Rasteniievodsto Imeni N. I. Vavilova, No. 22, pp. 35-38.

The authors claim resistance in S. chacoense and S. setulosisylum; the most resistant cultivars were in S. phureja and S. rybinii; but no form was immune.

Further work on resistance sources: at present not worth doing.

Ring rot. Resistance to ring rot has been claimed for some Tuberosum varieties and for wild species including S. acaule. It is resistance rather than immunity. There appears to be no recent work - this is presumably because ring rot can be controlled by seed growers using good cultural practices.

Further work on resistance sources: at present, not worth doing.

Brown rot or bacterial wilt - this is a serious disease where soil temperatures are high and there has been an interest in sources of resistance to Pseudomonas solanacearum. References:

- (a) THURSTON, H. D. and LOZANO, T. J. C. (1968). Resistance to bacterial wilt of potatoes in Colombia clones of Solanum phureja. American Potato Journal 45, 51-55.
- (b) SEQUEIRA, L. and ROWE, P. R. (1969). Selection and utilization of Solanum phureja clones with high resistance to different strains of Pseudomonas solanacearum. American Potato Journal 46, 451-462.
- (c) ROWE, P. R. and SEQUEIRA, L. (1970). Inheritance of resistance to Pseudomonas solanacearum in Solanum phureja. Phytopathology 60, 1499-1501.
- (d) FRENCH, E. R. (1973). Evaluación de la resistencia de clones de papa a Pseudomonas solanacearum. Phytopathology 62, 757-758 (Abstract).
- (e) GONZALEZ, L. C., SEQUEIRA, L., ROWE, P. R. and BIANCHINI, R. (1973). Field resistance to bacterial wilt in hybrid potato progenies. Phytopathology 62, 760 (Abstract).
- (f) ROWE, P. R., SEQUEIRA, L. and GONZALEZ, L. C. (1972). Additional genes for resistance to Pseudomonas solanacearum in Solanum phureja. Phytopathology 62, 1093-1094.

Although resistance has been claimed in occasional accessions of several species, recent work has been concentrated on using certain clones of the diploid, cultivated species, S. phureja. Resistance is apparently not common, e.g. 6 resistant in 1061 clones tested (ref. a). There are also races of the bacteria and potato clones may be resistant to all or only to one (ref. b). Resistance is due to a number of dominant genes plus modifiers (ref. c and f) and varies with soil temperature, varieties being more susceptible the higher the temperature (ref. d). It is possible to transfer the resistance to S. tuberosum progenies (ref. e) but the degree of resistance varies with the virulence of the bacterial race.

Further work: the obvious place to look for further resistance are any clones of S. phureja which have not been previously tested. Bacterial wilt is a serious disease in both India and Kenya; it might therefore be advisable to test resistant clones in both these countries. Obviously, there is a big

danger of the bacteria producing new races.

Resistance to Diseases caused by Fungi (including Actinomycetes)

Many fungi can produce diseases of potatoes and it has only been possible to consider the apparently more important diseases. Relatively uncommon diseases can, however, be important in restricted areas or become important if a new variety with otherwise very good characters is very susceptible.

Common scab (Streptomyces scabies) - widespread; spoils the appearance of tubers rather than reduces yield. Sources of resistance: common scab is one of the other fungi in Ross and Rowe (1969). Resistance has been claimed in a number of species, including S. tuberosum both ssp. tuberosum and ssp. andigena. There are several resistant Tuberosum varieties which can be used as a source of resistance; e.g. see

WENZL, H. (1962). Beiträge zur ökologie des Kartoffelschorfes (Spongospora and Actinomyces scab). Pflanzenschutzberichte 29, 33-64.

Resistance in Tuberosum varieties can be found in their dihaploids and is due to more than one gene:

CIPAR, M. S. and LAWRENCE, C. H. (1972). Scab resistance of haploids from two Solanum tuberosum cultivars. American Potato Journal 49, 117-119.

ALAM, Z. and PELOQUIN, S. J. (1971). Variation in scab reaction of 24-chromosome S. tuberosum clones and families. American Potato Journal 48, 301-302 (Abstract). A reference to resistance in potatoes other than Tuberosum is:

DIONNE, L. A., and LAWRENCE, C. H. (1961). Early scab resistant derivatives of Solanum chacoense x Solanum phureja. American Potato Journal 38, 6-8.

It should also be noted that there are aberrant types of Streptomyces scab to which varieties resistant to common scab are not resistant; fortunately they are rare in occurrence.

HARRISON, M. D. (1962). Potato russet scab, its cause and factors affecting its development. American Potato Journal 39, 368-387.

Further work: probably adequate sources of resistance to common scab in Tuberosum and Andigena potatoes. Techniques for tests of resistance also probably adequate - the influence of soil moisture on scab development is not well understood.



Powdery Scab (*Spongospora subterranea*) - although not listed by Ross and Rowe (1969), powdery scab can be a serious disease in certain wet areas and five recent references were found to sources of resistance:

DUTT, B. L. and PUSKARNATH (1960). Resistance of potato varieties to powdery scab. Indian Potato Journal 2, 78-82.

WENZL (1962) - see reference under common scab.

MANZER, F. E., AKELEY, R. V. and MERRIAM, D. (1964). Resistance to powdery scab in *Solanum tuberosum* L. American Potato Journal 41, 374-376

ZADINA, J. (1965). (Problems of breeding potatoes for resistance to *Spongospora subterranea* (Wallr.) Johnson). Genetika a Slechteni 38, 71-78.

KORDZINSKI, J. (1970). (Results of four years of investigation on the susceptibility of Polish potato varieties to powdery scab). Builetyń Instytutu Ziemiaka 1970 (No. 6), pp. 65-77.

Considerable resistance exists in *Tuberosum* varieties; there are reports previous to 1960 of resistance in other species.

Future work: probably not a disease of sufficient importance to warrant screening potato collections for resistance; breeders (e.g. myself) may have unpublished data on resistance; testing methods at present not adequate as depend on an infested soil and a wet season.

Wart (*Synchytrium endobioticum*) - the use of so-called wart immune varieties is often quoted as being one of the triumphs of plant breeding; certainly their use, coupled with legislative action, has reduced wart in many countries so much that it now presents a problem of little importance. There are no immune varieties, only varieties of very high resistance:

NOBLE, M. and GLYNNE, M. D. (1970). Wart disease of potatoes. FAO Plant Protection Bulletin 18, 125-135.

In certain restricted areas there are more than one race of wart and resistance to these is not so widespread as to the common race, e.g.

PROUDFOOT, K. G. (1971). Further observations on races of potato wart in Newfoundland. Potato Research 4, 232-233.

Sources of resistance: widespread in *Tuberosum* and *Andigena* potatoes; also known in cultivated diploids and wild species (other fungi No. 3 in

ROSS and ROWE, 1969).

Further work: not an important disease in many countries; probably Andigena potatoes will supply resistance to the uncommon races.

Blight (Phytophthora infestans) - There must have been more work done on blight disease of potatoes than on all other diseases of potatoes put together. It is also, of course, one of the classical examples of the failure of simple dominant gene-controlled, hypersensitive type resistance to give lasting protection because of the rapidity with which new races of the pathogen arise. The failure has given rise to breeding for the polygenically controlled "field resistance" which is expected to be durable and also, to a lesser extent, to breeding for resistance of tubers to infection.

The following 14 references have been chosen, not for comprehensiveness but to illustrate certain points:

- 1) NIEDERHAUSER, J. S. and COBB, W. C. (1959). The late blight of potatoes. Scientific American 200, 100-112.
- 2) CERVANTES, J. (1965). Late blight-resistance of nine Mexican potato varieties in ten years of field trials. American Potato Journal 42, 258 (Abstract).
- 3) LAPWOOD, D. H. (1971). Observations on blight (Phytophthora infestans) and resistant potatoes at Toluca, Mexico. Annals of Applied Biology 68, 41-53.
- 4) BLACK, W. (1970). The nature and inheritance of field resistance to late blight (Phytophthora infestans) in potatoes. American Potato Journal 47, 279-288.
- 5) UMAERUS, V. (1970). Studies on field resistance to Phytophthora infestans. 5. Mechanisms of resistance and applications to potato breeding. Zeitschrift fur Pflanzenzuchtung 63, 1-23.
- 6) YOUNG, R. J., DEAHL, K. L. and GALLEGLY, M. E. (1972). American Potato Journal 49, 365 (Abstract).
- 7) THURSTON, H. D., HEIDRICK, L. E. and GUZMAN, N. J. (1962). Partial resistance to Phytophthora infestans (Mont) de Bary within the Colección Central Colombiana. American Potato Journal 39, 63-69.
- 8) SIMMONDS, N. W. and MALCOLMSON, J. F. (1967). Resistance to

late blight in Andigena potatoes. European Potato Journal 10, 161-166.

- 9) ESTRADA RAMOS, N. and GUZMAN NARANJO, J. (1969). Herencia de la resistencia de campo al "tizón" (Phytophthora infestans (Mont.) de Bary) en variedades cultivadas de papa (subespecies tuberosa y andigena). Revista del Instituto Colombiano Agropecuario 4, 117-137.
- 10) DIONNE, L. A. and HODGSON, W. A. (1966). Advances in potato late blight resistance. Canadian Agriculture 2, 28-29.
- 11) GRAHAM, K. M. and DIONNE, L. A. (1961). Crossability relationships of certain diploid Mexican Solanum species. Canadian Journal of Genetics and Cytology 3, 121-127.
- 12) DIONNE, L. A. (1963). Studies on the use of Solanum acaule as a bridge between Solanum tuberosum and species in the series Bulbocastana, Cardiophylla and Pinnatisecta. Euphytica 12, 263-269.
- 13) GRAHAM, K. (1965). Experimental hybridization in certain diploid Mexican Solanum species. Euphytica 14, 113-119.
- 14) LANGTON, F. A. (1972). The development of a laboratory method of assessing varietal resistance of potato tubers to late blight (Phytophthora infestans). Potato Research 15, 290-301.

The breeding of varieties with field resistance to blight has benefited greatly from the work of Niederhauser and co-workers in the Toluca Valley. (ref. 1-3) - in addition to breeding varieties suitable for Mexico (ref. 2), it was possible for workers in other countries to send material for testing. Workers in other countries (e.g. refs. 4, 5 and 6) have added to the techniques necessary for testing for field resistance.

#### Sources of resistance:

Tuberosum potatoes - there is some field resistance present but it is small compared with that obtainable from Mexican wild species (ref. 6).

Andigena potatoes - most clones are more susceptible than Tuberosum varieties and a major difficulty in using Andigena potatoes as sources of resistance to cyst nematodes (Heterodera spp) is their marked susceptibility to blight. However it is claimed (ref. 7, 8 and 9) that some Andigena clones have a field resistance to blight higher than of many Tuberosum varieties.

Diploid cultivated potatoes - ref. 7 claims that some S. phureja accessions have a high field resistance - most, however, are very susceptible.

Wild potatoes - the best sources of field resistance appear to be the Mexican wild potatoes of which most use has been made of S. demissum and S. stoloniferum (these species, of course, also contain R genes). Attempts to use other wild species (S. pinnatisectum, S. cardiophyllum, S. bulbocastanum, for example) as sources of field resistance are more difficult to use (ref. 10-13) because of sterility barriers.

Future work: it can be argued that there are now sufficient sources of field resistance to blight available for use by breeders, and it would also seem that the wild Mexican source is better than Andigena, or S. phureja sources. The continuation of facilities such as Toluca valley for testing may be advisable. Another type of resistance, resistance of tubers to infection, has not been explored to any extent - one particular difficulty is a quick test, tests such as those of Langton (ref. 14) are not satisfactory. So far, the indications are that field resistance is stable and not eroded by the fungus producing new races (ref. 1 & 6). If the field resistance was not stable, there would obviously be a more urgent need to find new sources of resistance.

Early Blight (Alternaria solani) - resistance to early blight is listed in Ross and Rowe (1969) as No. 1 in other Fungi. It is recorded for wild species such as S. chacoense, S. commersonii and S. tarijense. There appears to be little recent work, an exception being

DOUGLAS, D. R. and PAVEK, J. J. (1972). The relationship of the susceptibility of different clones of potatoes to early blight foliage and tuber infection. American Potato Journal 49, 370 (Abstract).

Future work: it needs to be decided whether the disease is important enough to warrant much work on discovering sources of resistance. If it is, then there is a necessity for finding locations where testing can be carried out.

Tuber Rots (Gangrene; dry rot; charcoal rot).

In addition to tuber rots caused by bacteria, there are at least three fungi which can cause severe rotting of tubers. Their importance varies with country. Gangrene (Phoma exigua var. foveata) is a serious disease where potatoes are harvested under cold conditions. Dry rot (Fusarium caeruleum) can also be serious under temperature conditions. Charcoal rot (Macrophomina

phaseoli) is of particular importance in India.

Gangrene is not a disease mentioned by Ross and Rowe (1969). There are differences in susceptibility, for example:

BANG, H. (1972). Mottaglighet fur Phoma-rota i svenskt potatis-material. Vaxtskyddsnotiser, 36, 46-47.

No survey of S. American potatoes for resistance has been made. Tests for resistance have been worked out.

Fusarium Dry Rot is No. 2 of Other Fungi of Ross and Rowe (1969). Resistance has been claimed for some *Andigena* accessions. It is also known in some *Tuberosum* varieties, for example:

AVERS, G. W. (1972). Fusarium decay in potatoes. Canada Agriculture 17, 38-39.

Charcoal rot is important in India, see:

PUSKARNATH (1961). Potato breeding and genetics in India. Indian Journal of Genetics 21, 77-86.

DEVENDRA, SAHAI, DUTT, B. L. and PAHARIA, K. D. (1970). Reaction of some wild and cultivated potato varieties to charcoal rot. American Potato Journal 47, 427-429.

The latter authors found resistance in six clones of *S. chacoense*; some resistance is also found in *S. tuberosum*.

Further work: these three tuber diseases are only important in certain areas and it should therefore be left to the local potato breeding organizations to screen material for resistance.

Verticillium and Fusarium Wilts - Verticillium wilt is considered important enough by Ross and Rowe (1969) to be given a separate column in their lists. Fusarium wilt is No. 4 in Other Fungi.

Resistance to Verticillium wilt, (usually *V. albo-atrum*) has been found in many clones of *Andigena* (especially those from Colombia), in some clones of *S. phureja* and in several wild species (especially *S. chacoense* and *S. tarijense*). It also exists in a few potatoes grown in India (2 resistant and 31 tolerant out of 145 clones tested).

PHADTARE, S. G. and PUSKARNATH (1969). Occurrence of Verticillium wilt of potato in Simla hills and reaction of some commercial potato varieties to the pathogen. Indian Phytopathology, 22, 419-422.

It has been suggested that Peruvian potatoes contain useful germ-plasm for resistance to Fusarium wilts:

SEMINARIO, B., FRENCH, E. R. and NIELSEN, L. W. (1970). Resistencia de tubérculos a las Fusaria que afectan papa en el Perú. American Potato Journal 47, 118-123.

Further work: not immediately necessary to screen for more sources of resistance as present known sources probably not being used.

Other Fungi - there are still more fungi which can cause damage to potatoes. They include skin spot (Oospora pustulans), stem canker (Corticium solani), silver scurf (Spondylocadium atrovirens), pink rot (Phytophthora erythroseptica), watery wound rot (Pythium ultimum), stalk break (Sclerotinia sclerotiorum), black dot (Colletotrichum atramentarium), and violet root rot (Helicobasidium purpureum) in temperate regions and others such as a rust (Puccinia pitteriana) in Colombia and Sclerotium rolfsii wilt in India.

#### Resistance to Aphids

Aphids can on occasions cause direct damage to potato plants, but the main importance of aphid resistance is usually considered to be control of the aphid-transmitted virus diseases. There are three big reviews of aphid resistance:

RADCLIFFE, E. B. and LAUER, F. I. (1968). Resistance to Myzus persicae (Sulzer), Macrosiphum euphorbiae (Thomas), and Empoasca fabae (Harris) in the wild tuber-bearing Solanum (Tourn.) L. species. Technical Bulletin of Minnesota Agricultural Experimental Station No. 259.

RADCLIFFE, E. B. and LAUER, F. I. (1970). Further studies on resistance to green peach aphid and potato aphid in the wild tuber-bearing Solanum species. Journal of Economic Entomology 63, 110-114.

RADCLIFFE, E. B. and LAUER, F. I. (1971). An appraisal of aphid resistant tuber-bearing Solanum germplasm. Technical Bulletin, Agricultural Experimental Station, University of Minnesota No. 286, 24 pp.

Some of the above findings are also given in Ross and Rowe (1969). Resis-

tance to both aphids, the peach aphid (Myzus persicae) and the potato aphid (Macrosiphum euphorbiae), are primarily in certain Mexican wild potato species. Many of the resistant species are difficult to cross with cultivated potatoes. There is no resistance of even a low degree in cultivated potatoes. The resistance of wild species (S. polyadenium, S. tarijense, and S. berthaultii) which have glandular hairs that trap aphids has been investigated:

GIBSON, R. W. (1971a): Glandular hairs providing resistance to aphids in certain wild potato species. Annals of Applied Biology 68, 113-119.

GIBSON, R. W. (1971b). The resistance of three Solanum species to Myzus persicae, Macrosiphum euphorbiae and Aulacorthum solani (Aphididae: Homoptera). Annals of Applied Biology 68, 245-251.

Further work: again not necessary until more attempts have been made to use resources already discovered.

#### Resistance to Other Insects

In addition to aphids, Ross and Rowe (1969) list the reactions of accessions to leaf hoppers Empoasca spp and among Other Insects to flea beetle (Epitrix spp) and Colorado Beetle (Leptinotarsa decemlineata).

Resistance to potato leafhoppers was considered by Radcliffe and Lauer (1968-full reference in aphids section, see above). They found some resistance in Tuberous potatoes (there is also tolerance, e.g. in the USA var. Sequoia) and in a few Andigena clones, but much higher resistance in several wild Mexican species (e.g. S. bulbocastanum, S. pinratense and S. demissum). Resistance to leafhoppers has also been considered by:

SANDFORD, L. L. and SLEESMAN, J. P. (1969). Genetic variation in a population of tetraploid potatoes: response to the potato leaf-hopper and potato flea beetle. American Potato Journal 46, 436 (Abstract).

SANDFORD, L. L., CARLSON, D. V. and HIBBS, E. T. (1972). Genetic variation in a population of tetraploid potatoes: foliage resistance to oviposition of the potato leafhopper. American Potato Journal 49, 98-108.

It appears that selecting for resistance to leaf hopper would tend to increase susceptibility to flea beetle and vice versa. Resistance to flea beetle (No. 1 in Other Insects of Ross and Rowe, 1969) is found in many potato accessions including Andigena potatoes and many wild species (e.g. S. stoloniferum).

Before 1950 it was considered desirable to breed for resistance to

Colorado Beetle, particularly in Germany and eastern Europe. Although there are some recent publications on resistance to Colorado Beetle, it is not given a high priority now because of successful control by insecticides:

SCHWARZE, P. (1963). Über den Glykoalkaloidgehalt und die Zusammensetzung des Glykoalkaloidkomplexes in Nachkommen der Artkreuzung Solanum tuberosum x Solanum chacoense. Züchter 33, 275-281.

BUKASOV, S. (1972). (Breeding potato varieties resistant to Colorado beetle). Kartofel i Ovskchi 11, 6.

Several species have resistance to Colorado beetle, the most important of which is probably S. chacoense. There is a danger of resistant plants owing their resistance to high contents of alkaloids.

Further work: it does not seem that insect resistance, with the possible exception of leafhopper resistance, warrants any further work at the present time.

#### Resistance to Cyst Nematodes

The nematodes which feed on potatoes were considered in detail at a CIP Working Party held in February 1974. The writer of these notes has seen the program of the meeting, but not any papers prepared for it nor any recommendations made. Potato nematode problems are discussed here briefly because they have implications as to how work on other pests and diseases should be carried out. Also it should be noted that resistance to cyst nematodes would be among the top priorities if British, Dutch and West German breeders were asked to indicate what was the most important genetic resource they are interested in.

The modern work on breeding potatoes resistant to cyst nematodes starts with the work of:

ELLENBY, C. (1954). Resistance to the potato root eelworm, Heterodera rostochiensis Wollenweber. Nature, London 170, 1016.

Dr. Ellenby, who was a lecturer at Newcastle University, England, screened the Commonwealth Potato Collection for resistance to potato cyst nematode (the golden nematode of the USA) and found resistance in the wild species, Solanum vernei, and 5 clones of Andigena potatoes in some 1,000 accessions. It should be noted that Dr. Ellenby had no official connection with the CPC. His test for resistance was simple-potatoes were planted in a nematode-infested soil and their root-balls examined at an appropriate stage. Plants



with many cysts were susceptible, plants with no, or few, cysts were resistant. This simple test, which could be applied to many hundreds of selections a year, if necessary, is still that used by practical breeders, and its simplicity must have contributed much to the success in Europe and the USA in breeding resistant varieties in a relatively short time.

The second factor leading to quick progress in breeding resistant varieties was that the source of resistance used was in *Andigena* potatoes (usually CPC 1673) and was due to a single dominant gene.

The third factor which made success relatively easy was that in much of Europe there is only a single pathotype of *Heterodera rostochiensis* present - the extreme example is in Sweden where of 600 populations tested only one was capable of producing many cysts on resistant potatoes bred from the *Andigena* source:

VIDEGARD, G. (1969). Nematodresistenta sorter - saneringseffekt och faran fur resistensbrytare. Potatis 1969, pp. 26-28.

The situation is presumably due to only a very small number of cysts being introduced into Europe from S. America, and it has been found that European resistant varieties are not resistant to many S. American nematode populations:

MAYER de SCURRAH, M., MAI, W. F. and PLAISTED, R. L. (1973). More about the potato nematode, *Heterodera rostochiensis* Woll. in Peru. American Potato Journal 50, 58-61.

MAYER de SCURRAH, M. (1972). Variability in *Heterodera* attacking the potato in Peru. In Prospects for the potato in the developing world, Ed. E. R. French. Lima: CIP pp. 172-180.

It is now known that there are in Europe apparently two species of cyst nematode, *H. rostochiensis* sensu stricto and *H. pallida*. There are also more than one pathotype of each species. The Ellenby *Andigena* resistant material (e.g. CPC 1673) is only resistant to pathotype A of *Heterodera rostochiensis*; hence attention has had to be given to other sources of resistance including *S. vernei* which was already known to be resistant:

- 1) ROSS, H. and HUIJSMAN, C. A. (1969). Uber die Resistanz von *Solanum (Tuberarium)*, Arten gegen europaische Rassen der Kartoffelnematoden (*Heterodera rostochiensis* Woll.). Theoretical and Applied

Genetics 39, 113-123.

- 2) DESHMUKH, M. G. and WEISCHER, B. (1970). Resistance of wild species of potato to populations of Heterodera rostochiensis Woll. from West Germany. Potato Research 13, 129-138.
- 3) BOUWMAN, L. A. and ROSS, H. (1972). Differentiation between Heterodera rostochiensis and an undescribed allied species by female colour, morphometrics and pathogenicity. Nematologica 18, 265-269.
- 4) HUIJSMAN, C. A. (1972). Wilde en primitieve Solanum - soorten en aardappelmoeheids-resistentie. Zadbelangen 26, 228-229.
- 5) PLAISTED, R. L., SCURRAH, M. M. de and HARRISON, M. L. (1972). Resistance to the potato nematode Heterodera rostochiensis Woll. in clones derived from Solanum vernei. American Potato Journal 49, 364 (Abstract).
- 6) HOWARD, H. W., COLE, C. S. and FULLER, J. M. (1970). Further sources of resistance to Heterodera rostochiensis Woll. in the Andigena potatoes. Euphytica 19, 210-216.
- 7) ROTHACKER, D. and STELTER, H. (1971). Solanum tuberosum ssp. andigenum als Resistenzquelle für die Nematodenresistenzzüchtung. See Plant Breeding Abstracts 42, Abstract 5481.
- 8) HUIJSMAN, C. A., KLINKENBERG, C. H. and OUDEN, H. den (1969). Tolerance to Heterodera rostochiensis Woll. among potato varieties and its relation to certain characteristics of root anatomy. European Potato Journal 12, 134-147.

The above 8 references are not comprehensive but have been chosen to illustrate certain aspects. Sources of resistance to Heterodera pallida and H. rostochiensis:

Tuberosum potatoes	-	Chile EBS 2084 (ref. 1 only).
Andigena potatoes	-	CPC 2775, 2802 & 2805 (ref. 6 only).
Cultivated diploids	-	none

Wild species - S. andreanum (ref. 1); S. boliviense (ref. 2); S. brevicaule (ref. 2); S. kurtzianum (ref. 3) S. leptophyes (ref. 4) S. megistacrobium (ref. 1 & 2); S. multidissectum (refs. 4 & 6); S. oplocense (refs. 1, 3 & 4); S. sparsipilum (refs. 1, 2 & 4); and S. vernei (refs. 1, 2, 3, 4 & 5). There may also be resistance in S. acaule and S. chaco-

ense (ref. 4).

The most widely investigated of the sources of resistance to H. pallida and H. rostochiensis (all, not only A, pathotypes) is S. vernei. It is interesting to note that its resistance was thought to be non-race specific and to be caused by polygenes; more recent work (ref. 4) suggests it may be due to a series of major genes which are to some extent at least race-specific. S. vernei may even contain a major gene similar to that found in Andigena CPC 1673 (ref. 5). It has been suggested that various wild species are the best source of resistance e.g. S. sparsipilum or S. oplocense.

The Andigena resistance (ref. 6) was found in 5 lines which traces back to 3 accessions only in screening 310 self or sib-crosses and 370 crosses between clones. The resistance was thus rare - compare Ellenby's results but contrast with ref. 7 in which no fewer than 68 forms of Andigena were found to be resistant to pathotype A of H. rostochiensis - the latter may be a false result due to testing a short day species under long days (see also page 6).

It is possible that no source will be found with resistance to all pathotypes of all cyst nematode species; this would apply specially to S. America. In such circumstances tolerance to nematode attack could be valuable. Tolerance may exist (ref. 8).

Further work: work in progress may show that sources of resistance adequate to cover pathotypes of cyst nematodes present in Europe already exist; the problem in S. America is more complex and needs further investigations which are being carried out. Nematode resistance is important as an indirect method of increasing potato yields.

#### Resistance to Other Nematodes

Resistance to root-knot nematodes, Meloidogyne species may be important in warmer soils. Meloidogyne species are Other Nematodes No. 1 in Ross and Rowe (1969). Reactions to Meloidogyne have been investigated by:

BRUCHER, H. (1967). Root knot-eelworm resistance in some South American tuber-forming Solanum species. American Potato Journal 44, 370-375.

NIRULA, K. K., NAYAR, N. M., BASSI, K. K. and SINGH, G. (1967). Reaction of tuber-bearing Solanum species to root-knot nematode, Meloidogyne incognita. American Potato Journal 44, 66-69.

NIRULA, K. K., KHUSHU, C. L. and RAJ, B. T. (1969). Resistance in tuber-bearing Solanum species to root-knot nematode, Meloidogyne incognita. American Potato Journal 46, 251-253.

Resistance to M. incognita and other species is found in many species, but no work on the inheritance of resistance appears to have been published.

Potatoes are also attacked by a race of the stem nematode (Ditylenchus dipsaci) and resistance has been claimed:

GERMAN, E. (1972). (Resistance of potatoes to the stem nematode). Kartofel' i Ovoshchi, No. 40 p. 40.

There are also many migratory nematodes attacking potato roots. It is unlikely from comparison with work on other crop plants, that any resistance to these occurs.

Further work: there may be a case for more studies of resistance to Meloidogyne.

Yield. Although in potatoes breeding for disease resistance is often the easiest way of increasing yields, it may be possible by using in breeding programs new sources of genetic variation to find hybrid vigour and to increase yields.

The cultivated diploid potatoes and many of the wild S. American diploid species are outbreeders with a well developed S allele system. Also, although the cultivated tetraploids are self-compatible, studies of their dihaploids have shown that they possess S alleles similar to those in the cultivated diploids. It may be that self-compatibility in Andigena and Tuberosum potatoes is due to competitive interaction of S alleles (i.e. S<sub>1</sub> S<sub>2</sub> pollen can function in S<sub>1</sub> S<sub>1</sub> S<sub>2</sub> S<sub>2</sub> styles). The Andigena and Tuberosum potatoes may therefore behave as outbreeders and not self-pollinators, particularly so far as vigour of offspring is concerned.

There is some evidence for hybrid vigour in tetraploid potatoes:

- 1) ROTHACKER, D. (1962). Populationsanalytische Untersuchungen über die Leistung verschiedener Kreuzungskombinationen zwischen ssp. andigena und ssp. tuberosum von S. tuberosum. European Potato Journal 5, 1-13.
- 2) GLENDINNING, D. R. (1969). The performance of progenies obtained by crossing groups Andigena and Tuberosum of Solanum tuberosum. European Potato Journal 12, 13-19.

- 3) HANNEMAN, R. E. and PELOQUIN, S. J. (1969). Use of Phureja and haploids to enhance the yield of cultivated tetraploid potatoes, American Potato Journal 46, 436 (Abstract).
- 4) MENDIBURU, A. and PELOQUIN, S. J. (1971). High yielding tetraploids from 4x-2x and 2x-2x matings, American Potato Journal 48, 300-301. (Abstract).

Care is needed, however, in measuring hybrid vigour in potatoes; in particular it is necessary to compare clones of similar maturity.

Further work: because the Tuberosum varieties now used by breeders tend to be hybrid derivatives involving a wider range of parents than the Tuberosum varieties bred before 1940, it is possible that newer introductions may have some hybrid vigour. For S. America it might be interesting to study crosses between Andigenas from the different ends of its range.

#### Discussion

This survey of genetic resources, which is not completely comprehensive, suggests four major conclusions:

1. There is in the tuber-bearing Solanum species a vast store of germ plasm in both the cultivated and wild species.
2. There is a wide range of diseases and pests of varying importance.
3. Resistance to some diseases and pests may be found in either the cultivated tetraploids or the cultivated diploids but for certain pathogens resistance is only found in the wild species.
4. Even when resistance has been found, it is not being used to any extent in breeding programs.

## CONSERVATION OF GENETIC RESOURCES

### Introduction

There are already in existence large collections of both cultivated and wild potatoes, and plans have been made (CIP Workshop on Germ-Plasm Exploration and Taxonomy of Potatoes, January 1973, Lima) to assemble even more material by a number of collecting expeditions between 1973 and 1977. The Workshop also suggested that the first priority should be the collection of cultivated forms and that the first priority in taxonomic research should be given to cultivated forms. The last four recommendations of the ten they made were:

"7. In relation to conservation work the workshop stressed that if the plans outlined in this paper come to fruition that adequate facilities be made for the expanded amount of material which the gene bank will have to hold.

8. Further, it was stressed that the bank should possess as broad a base as possible of material in the form of true seed. Research on the techniques to be used to convert the cultivated forms to true seed should be conducted as soon as possible.

9. In addition, it was recognized that a limited number of cultivars, breeding lines and genetic stocks should be maintained clonally for a number of years, for demonstration and research purposes. Certain odd-number polyploids may also need to be maintained clonally.

10. Finally, the workshop suggested, although conceding that this was beyond its brief, that there was an urgent need to increase efforts in evaluation to facilitate the utilization of the material for the needs of developing countries".

In my opinion, these four recommendations will be very difficult to carry out and they need careful consideration. Considering the first three recommendations, it can be added that the CIP has about 4000 cultivated forms and expects to receive another 2000 to 4000 forms (P. R. Rowe in a letter discussing this workshop). I suggest:

1. That as much as possible of the material should be put immediately into true seed for at least three reasons:

(a) Maintaining material clonally is a time - and labour-consuming activity which is of little interest to those who do it.

(b) In spite of precautions, clones very quickly pick up virus diseases and the process tends to accelerate the longer the material is kept as clones.

(c) It is easier to distribute material as true seed and true seed causes very much less trouble from quarantine restrictions than do tubers.

2. It is too late to do the research on techniques to convert the cultivated forms to true seed. Fortunately, the few techniques needed are already known and it should not be difficult to get most of the material into seed. Material that cannot be converted into true seed should be discarded - this may be contrary to what some conservationists would do, but it is not worth the time to keep such types when there is an abundance of more amenable material.

3. Evaluating the material cannot be organized very quickly and it needs experts to do it. Hence the material will have to be kept for many years before it can be evaluated.

### Storage of True Seed

Once converted into true seed, material can be kept with very little trouble for many years. It appears to be a general rule for most species:

HARRINGTON, J. F. (1970). Seed and pollen storage for conservation of plant gene resources. In O. H. Frankel and E. Bennett (Editors). Genetic Resources in Plants: their Exploration and Conservation. Oxford: Blackwell Scientific Publications, pp. 501-521.

that the life of seeds is doubled for every 5°C drop in temperature. Potatoes are probably no exception to this rule and, although

SIMMONDS, N. W. (1968). Prolonged storage of potato seeds. European Potato Journal 11, 150-156.

suggests a limit of 12 years for low-dormant diploids and 16 years for deeply dormant tetraploids, the limits may be much longer using storage at 5°C (a temperature readily available in low cost refrigerators). Two other references:

ROSS, R. W. (1969). Seed dormancy and longevity in Solanum species. American Potato Journal 46, 438 (Abstract).

HOWARD, H. W. (1969). The storage of true seeds of potatoes. European Potato Journal 12, 278-279.

both suggest 15 years as a minimum for storage and that 20 years or even more may be possible (the Howard experiment reaches 20 years in 1974). Seeing that seed stored at room temperature (about 15°C) can be viable for at least 10 years, storage at 5°C may enable seed to be kept for 40 years. Even with a limit of 15 years, the advantages of keeping a collection as true seed are obvious.

The mechanics of keeping the collection as true seed requires a little consideration. It is of some advantage not to keep the seeds as big bulks but to divide them into 10 or so small bulks. When a distribution has to take place, it causes less disturbance to take one of the small packages out of store than to have to take a big bulk out, separate out a part of it for distribution, and then return the bulk to the cold store.

### Pollen Storage

Pollen can be stored for two years at -20°C (deep freeze conditions) and such stored pollen can be very useful for pollinating pollen-sterile clones, particularly as it obviates having the pollen-fertile clone in flower at the same time as the pollen-sterile:

KING, J. R. (1955). Irish potato pollen storage. American Potato Journal 32, 386-391.

HOWARD, H. W. (1958). The storage of potato pollen. American Potato Journal 35, 676-678.

### Induction of Flowering

Clones which do not flower can often be made to flower by either grafting scions on to tomato stocks or by "growing on a brick":

THIJN, G. A. (1954). Observations on flower induction with potatoes. Euphytica 3, 28-34.

### Cultivated Tetraploids - Tuberosum potatoes

Many clones are pollen-sterile and the only way of keeping them as true seed must involve making crosses with pollen-fertile clones. Doing this, however, results in valuable characters due to polygenes being lost. A few varieties are also more or less ovule-sterile. It is, however, no real loss to lose such types as they are of little use in breeding programs because of their sterility.

The Chilean tetraploid potatoes have not been studied to any extent and they may pose more problems in getting them into true seed than do the tetraploid cultivated Andigena potatoes.



### Cultivated Tetraploids - Andigena potatoes

The preservation of the germ plasm present in the Andigena potatoes is a major problem for the CIP. On the whole there is no lack of pollen-fertile clones but not every clone is pollen fertile. For example, of the 218 Andigena accessions received by the IR-1 Potato Collection as tubers, only 29 were available as clones, 16 as open-pollinated seed, 74 as selfed seed and 99 as hybrid seeds in 1969.

On the whole selfed seed is to be preferred to hybrid seeds. If a desirable character is due to a single dominant gene, then there is little advantage for selfed over hybrid seed. On the other hand, if the desirable character is due to a number of polygenes, selfed seed has a big advantage over hybrids for finding the desirable character again in the progeny.

Where selfed seed cannot be obtained and resource has to be made to producing hybrid seed, then it would presumably be advantageous to use as a pollinator, another clone from the same region. When it is necessary to screen an Andigena collection which has been put into true seed, it is useful to have progenies from selfed seed of the pollinators to compare with the progenies from crosses.

A further problem which will have to be considered in the future is what to do when the primary seed is reaching the end of its viability. The only process possible is sib-crossing for accessions preserved either as selfed or hybrid seed. How many sibs should be included in producing the new seed stock has never been considered. Space, time and labour will, however, set a limit to the number.

As suggested earlier, accessions from which seed cannot be obtained should be discarded. They are of no use to the potato breeder and, if preserved as clones, will in the end be a dangerous source of virus diseases.

### Cultivated Triploids and Pentaploids

There seems to me to be no reason to conserve the few pentaploid S. curtilobum clones. It can be argued that these clones are unlikely to contain valuable genes not found in the cultivated tetraploids and diploids and the wild species, S. acaule.

The two triploid "species", S. chaucha and S. juzepczukii, can be treated with colchicine to produce hexaploids which may be fertile:

HOWARD, H. W. (1961). The production of hexaploid Solanum x juzepczukii, Euphytica, 10, 95-100.

It would, however, be a difficult task to convert many triploids to hexaploids, and, as with the pentaploids, there are probably few, if any, valuable genes in the triploids which cannot also be found in the cultivated tetraploid and diploid potatoes and S. acaule.

The one feature which the triploid and pentaploid cultivated "species" may have is either resistance to or tolerance of virus diseases. If they are tolerant, this increases the danger of preserving them as clones from which the rest of a collection can become infected.

#### Cultivated Diploid Species

The cultivated diploid potatoes are self-incompatible and hence must normally be conserved as hybrid seed. Some can be selfed, either by bud-pollination or at the end of the season. But, as vigour is often lost on inbreeding, there is no doubt that they should be preserved as hybrid seed. Again the hybrid seed should be produced between clones from the same region.

#### Wild Species

These obviously should be conserved as seed.

### SCREENING FOR VALUABLE GENES

There are two main problems to consider when considering the problem of screening for valuable genes. These are first what genes are wanted and secondly, where is the screening to be done.

The purpose of the major part of these notes - Survey of Genetic Resources - was to consider which genes are wanted, which genes have already been found, and what are the problems in utilizing these genes in practical breeding programs. One important conclusion was that it would be advisable to draw up an order of importance for screening for characters wanted in new varieties in the very many countries where potatoes are an important crop and in countries where they may become more important.

It seems obvious to me that it is unlikely that any single organization can do all the screening that is wanted. It was also pointed out earlier that for some diseases and pests there exist fewer pathotypes in many countries than in South America and hence that screening in South America might be much more severe than in the rest of the world. It would,

therefore, be often advisable that the screening should be done in close contact with the breeders in any countries or even by the breeders themselves.

On the other hand, it is obviously wasteful of effort for screening to be going on in very many places and it would often be impractical because of a lack of enough specialized pathologists. Not all pathologists have been trained to consider resistance to pathogens in a way of value to plant breeders and they do not always appreciate the difficulties of breeding new varieties. Neither do they realize that a moderate degree of resistance which it may be possible to breed for relatively easily must be the answer to a problem at present rather than a very high degree of resistance or immunity which it may be very difficult for breeders to incorporate in new varieties. There is a much better chance of useful screening being done if the pathologists are in close contact with the breeders.

It is not obvious to me how a co-operative scheme of screening potato collections should be organized but it is on the other hand obvious that it should be the task of the CIP to consider the problem. An interim measure could be for the CIP to produce a "Potato Newsletter". The first issues of this would contain a request for countries to outline what they considered were the most important characters required in new varieties; whether they had adequate sources from which to obtain these characters, and, if not, were they screening for them; and what facilities they had or intended to provide for such screening. Later it should be possible to publish lists of new stocks with valuable characters which were available in different countries.

Finally, it must be admitted that putting a collection into true seed can make screening for valuable genes more difficult. Consider, for example, a required character which is controlled by a single dominant gene. If selfed seed has to be screened, then three in four of the progeny will on average have the desired character; if hybrid seed has to be screened, then it is only one plant in two. This necessitates growing about five plants per progeny. The screening may also take a year longer as it often cannot be done satisfactorily with seedlings and needs plants grown from tubers.

### GENERAL REFERENCES

(i.e. those not given in the various parts of the Section Genetical Resources)

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## APPENDIX 2

### PROPOSED SCHEME FOR POTATO PRODUCTION FROM BOTANICAL SEED

The proposal is based on the use of sexual production of tetraploids via  $2n$  gametes produced by first meiotic division restitution (FDR) in diploid clones ( $4X$  or  $2X \times 2X$  crosses).

#### Advantages

- Reasonably high level of uniformity by generating a homogeneously heterozygous population.
- Control of virus diseases even in the absence of a sophisticated certification seed program.
- Utilization of heterosis by making unrelated hybrid clones in the production of seed.

#### Method

- Identify diploid clones that combine good agronomic characteristics with production of  $2n$  gametes by FDR.
- Identify good combinations, i.e., clones that produce agronomically acceptable, mainly tetraploid, progeny efficiently. These means that:
  1. they "nick" well
  2. produce  $2n$  gametes in reasonably high numbers

If a female diploid parent with the required characters is not available, a male sterile tetraploid clone could be used as female in a  $4X \times 2X$  cross.

#### Procedure

Establish a crossing plot by planting the parents in the field. For example, 3 rows of the female  $4X$  parent to one of the male parent.

The crossing plot should be isolated from other potato pollen sources.

Harvest fruits (only produced by the female parent).

The use of two diploid clones would be advisable if

- 1)  $2n$  gametes are produced by FDR in both sexes,
- 2) the proportion of  $4X$  progeny is high and
- 3) the  $2X$  progeny is easily identified and discarded.

### APPENDIX 3

Prepared by the Secretariat in the Crop  
Ecology and Genetic Resources Unit, FAO

February 1974

#### The International Board for Plant Genetic Resources - its foundation and relationship to other international agencies

The purpose of this brief background paper is to inform you of the events that have led to the establishment of this International Board for Plant Genetic Resources. An outline is presented of the representation, and important future functions and responsibilities of the Board.

#### What is the Board?

It is a group of eminent scientists and administrators, the major regions of the developing world and FAO represented amongst its membership. Also included are representatives of donor organizations and governments with expertise in the various disciplines required for its future task, for example, plant breeding, cytology, taxonomy, horticulture and forestry. FAO has provided the headquarters for the Board in Rome and its Secretariat in the Crop Ecology and Genetic Resources Unit of FAO's Plant Production and Protection Division.

#### What has the Board to do?

Its basic purpose is to promote and support international, regional, and national actions in the collection and conservation of the world's plant genetic resources, recommending the necessary financing requirements to the Consultative Group on International Agricultural Research. How will this enormous task be accomplished? Through the development of a worldwide network of institutions and centres, the Board will identify with the assistance of FAO the needs for exploration, collection, conservation and evaluation of plant genetic resources. It will strengthen the relevant programmes of existing institutions and, where appropriate, encourage the implementation of expanded activities; support training programmes and the dissemination of information and material among institutions and centres in the network.



Which organizations have participated in the establishment of the Board?

Two international organizations have been involved in the negotiations which have led to the establishment of the Board. The main sponsor of the Board is the Consultative Group on International Agricultural Research and a brief description of the Group's activities is given below. The Food and Agriculture Organization of the United Nations (FAO) has also had a major role in the Board's foundation due to its past and present activities in the field of plant genetic resources and the international nature of FAO's programmes.

Consultative Group on International Agricultural Research. -

The Consultative Group on International Research (CGIAR) was founded in early 1971 on the initiative of the World Bank, FAO and the United Nations Development Programme (UNDP) which are joint sponsors of the Group. Its members include governments, private foundations, development banks and representatives of the five major developing regions of the world. CGIAR has its headquarters in Washington, U. S. A. its main purpose is to mobilize long-term financial support from international agencies, governments and private sources for financing international agricultural research programmes and institutions. For instance, CGIAR supports the development of international centres, for example, CIMMYT in Mexico, CIAT in Colombia, ICRISAT in India, etc.

Technical Advisory Committee. -

The Consultative Group constituted a Technical Advisory Committee (TAC) in 1971 to support its activities. TAC's terms of reference are "to advise the Consultative Group on the main gaps and priorities in agricultural research related to the problems of developing countries; to conduct feasibility studies on the conduct of such research; to make recommendations for action to the Consultative Group; taking such studies into account; to advise on the effectiveness of existing international agricultural research programmes and in every way to encourage the creation of a sound international agricultural research network with an adequate information exchange system".

The meetings of the TAC are usually held twice a year and are attended by, amongst others, the representatives of the different international centres. FAO provides the Secretariat for the TAC which enables additional expertise to be made available as required.

The activities of FAO in plant genetic resources. -

FAO's activities in genetic resources date from 1961, when FAO's Plant Production and Protection Division held a Technical Meeting on Plant Exploration

and Introduction to review the situation and make specific recommendations. This was followed by other conferences held in 1967 and 1973, jointly with the International Biological Programme. FAO was involved in the genetic resources centre established at Ismir, Turkey, in 1964, with support from UNDP and the Turkish Government. In 1968 the Crop Ecology and Genetic Resources Unit was established in the Plant Production and Protection Division of FAO.

Since that time the activities of the Unit have included the support of collecting mission, seed exchange, surveying the crops and areas most endangered by genetic erosion; publishing these surveys, genetic resources registers and the Plant Genetic Resources Newsletter. The Unit has also participated in the preparations made for the Beltsville meeting which prepared an outline for a global network of genetic resources centres.

A source of scientific and technical advice to the Genetic Resources Unit and to FAO has been the FAO Panel of Experts, selected to serve in their individual capacities, on Plant Exploration and Introduction. This was established in 1966 and there have been six meetings of this Panel. Serving in a similar way by providing scientific advice to the Forest Management Branch of FAO, a Panel of Experts on Forest Gene Resources was established in 1968. This Branch of FAO's Forestry Department has also produced, since 1972, a publication on Forest Gene Resources Information.

In November 1972 the Consultative Group had asked FAO to accept responsibility for coordinating genetic resources work should an international programme be adopted. The Director-General of FAO, therefore, proposed to the 17th Session of the FAO Conference, in November 1973, that additional funds be made available in the Regular Programme for the 1974/75 biennium to strengthen the coordinating function of the Crop Ecology and Genetic Resources Unit.

The Conference, comprising all the member nations of FAO, welcomed and strongly supported the very high priority given to strengthening the Unit to permit to coordinate an effective world-wide programme. In addition, the Conference endorsed the recommendation that FAO should provide headquarters facilities for the Board, as well as the location of the Board's Secretariat in the Crop Ecology and Genetic Resources Unit. It further requested that the FAO council should be kept informed of the Board's activities.

#### The foundation of the International Board for Plant Genetic Resources. -

CGIAR, IAC and FAO have discussed, during the past three years, the best method for implementing a broadly-based programme on a global scale for the conservation, exploration, documentation, evaluation and utilization of plant genetic resources as well as related training programmes. A meeting of experts,

sponsored by TAC, was held at Beltsville, U. S. A. in March 1972 at which these programmes were discussed.

At its sixth meeting in July/August 1973, TAC recommended to CGIAR that the proposals it had submitted should be financed. It was further suggested that CGIAR might wish to consider establishing an additional body which could, with the necessary approvals, be fitted into the FAO framework. Thereby, potential donor organizations and developing countries could be jointly associated with the development of such a global genetic resources proposal.

The CGIAR therefore, established in August 1973 a Sub-Committee on Genetic Resources. Its role was to determine the possibility of establishing an appropriate body and to consider the planning and execution of the activities in the proposal approved by TAC. At the first Sub-Committee meeting held in Rome in October 1973, the terms of reference of the International Board for Plant Genetic Resources (IBPGR) and its mode of operation were agreed. These were subsequently approved by the CGIAR at its meeting in November 1973.

At the second meeting of the CGIAR Sub-Committee on Genetic Resources, held in Rome on 6-7 February 1974, nominations were carefully considered and, subject to their willingness to serve, thirteen members were elected to the Board. Arrangements were discussed whereby the contributions of donor governments and organizations could be paid into a Trust Fund, held by FAO and made available to the Board for financing the action programmes approved by the Board. It was further agreed that the Secretariat of the Board would be in the Crop Ecology and Genetic Resources Unit. The financing of the Secretariat would be suitably arranged on an ad hoc basis until the formal establishment of the Board at its first meeting in June 1974.

**3**

***Late Blight***

## CONTENTS

	<u>Page</u>
I. INTRODUCTION	143
II. RESISTANCE TO LATE BLIGHT	144
III. SOURCES OF RESISTANCE	147
IV. BREEDING METHODS	149
V. THE FUNGUS	151
VI. ADAPTATION TO DIFFERENT ENVIRONMENTAL CONDITIONS	153
VII. ASSISTANCE TO NATIONAL POTATO IMPROVEMENT PROGRAMS	154
VIII. PROPOSED PROGRAM	155
IX. ADDITIONAL COMMENTS	159
APPENDIX I - Position Paper for the Late Blight Project Planning Conference	167

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## I. INTRODUCTION

Late blight of potato has been the most feared and destructive disease of this basic food crop since the disease was discovered more than a century ago. It is among the most important of all plant diseases and perhaps has received as much attention through research and extension as any. In spite of the great state of knowledge concerning it, late blight remains as a major problem for control in developed countries, and, more importantly, as a limiting factor in potato production in the emerging nations, the target for the major thrust of the International Potato Center.

The causal fungus, Phytophthora infestans (Mont.) de Bary, is biologically adapted to thrive in most environments where the potato is cultured. It is well adapted for widespread dissemination through the air or through movement of infected tubers of its host. The infected tuber is the major source of survival in developed countries, whereas in developing countries the continuous overlapping culture of the potato and tomato serves as an additional important means of survival.

Disease control measures include such practices as destruction of discarded infected tubers and volunteer plants, regulatory laws requiring seed tubers and tomato transplants to be free of disease, protection of foliage by application of fungicides, and the use of resistant varieties. Fungicidal control has developed to a high state of technology in many nations. The modern fungicidal chemicals and sophisticated but expensive machinery provides effective control in most developed nations. In some, resistant varieties are being introduced whereas in other developed nations resistant varieties have not been accepted because of inferior tuber quality or because the type of resistance available is not effective in the face of new races of the pathogen.

In many developing nations there is relatively little use of fungicides. Most of the culture in these countries consists of small plantings by subsistence farmers. Effective modern spray machines are usually too expensive and are not designed for use in this type of culture. Hand sprayers are used by some small growers but control is unsatisfactory in the rainy seasons when the potato is usually grown in tropical countries. More importantly, the subsistence farmer seldom has money to purchase sprayers and fungicides where they are available.

It thus remains that the greatest promise for control of potato late blight in developing countries lies with the introduction of resistant varieties. The knowledge that high levels of resistance to all known races of the pathogen are available gives confidence that this promise can become a reality. It is the firm belief of this planning group that the program of CIP for control of late blight should be aimed directly toward the establishment of stable resistant varieties in developing nations.



This report summarizes our view of the present status of resistance to late blight and recommends a research and development program which we believe can significantly reduce the importance of late blight in developing nations in a relatively short period, and provides for the development of the scientific base necessary for continued improvement in the future.

## II. RESISTANCE TO LATE BLIGHT

### Status of Knowledge

Research leading to the current state of knowledge of late blight has revealed that two genetic systems control host resistance to the disease. The system giving hypersensitive resistance is controlled by a series of at least eleven genes (R-genes) derived from S. demissum, each independently inherited as a simple Mendelian character with resistance being dominant. History has well recorded that as fast as these genes have been isolated individually or recombined in S. tuberosum clones, the fungus has produced new races to completely overcome them. It is obvious that this type of resistance alone will be of little or no value for stable resistance to late blight in developing countries.

The other genetic system is controlled by a series of multiple genes which appear to be additive in effect and inherited in a polygenic fashion. This has been termed 'field resistance' and is apparently stable in the face of the variable pathogen. It consists of a complex of factors including resistance to entrance of the fungus, resistance to growth of the parasite, and sporulation capacity, although additional factors play a role in field resistance. Field resistance is race non-specific. It is strongly influenced by physiological and environmental factors. This type of resistance offers much hope in international control of late blight.

Since field resistance is controlled by many factors, rather than one or two, the fungus is probably unable to adapt itself to cultivars possessing this kind of resistance, at least not easily for field resistance appears to be stable so far. Observations in Kenya and Colombia indicate that no change has occurred even after 20 years of cropping twice a year. In Mexico, however, some suggestions of possible increase of the pathogen's ability to overcome field resistance are present among Mexican cultivars; thus the variety Atzimba appears to be more susceptible than when it was selected. In addition, records of blight incidence from fungicide treated and untreated plots of many years ago, and those recently recorded, indicate that some clones may be slightly more affected. The possibility that a shift towards a greater capacity of the late blight fungus to overcome field resistance is taking place is of concern for the development of resistant varieties.

Little is known about the levels of resistance needed in the developing world, the adaptability of resistant selections to different growing conditions, and the influence of these conditions on the expression of field resistance. Standard differential host sets for field resistance, and for R-genes, would be useful tools to gain this needed information, as also is the development of standard disease assessment methods for comparisons of late blight readings in different locations and for differing purposes (breeding, variety testing, studies on late blight epiphytotics and on the mechanisms of resistance).

Although much has been learned about the mode of action of field resistance, the true nature of resistance is not understood. Further studies on the mechanisms of resistance and inheritance of resistance with emphasis on their different components would be of much benefit for the development of late blight resistant varieties. Such studies should be intensified and can probably be most efficiently done through linkage projects or by visiting scientists and post-doctoral fellows.

Currently foliage resistance is recognized as the most important goal to achieve for late blight control in developing countries. However, tuber resistance should not be neglected and a better understanding of various aspects of tuber resistance is needed. Tuber susceptibility should be taken into account in selection work at time of maturity. For the problem of tuber susceptibility consideration should be given to cultural practices that may be easier to achieve by subsistence farmers than in large scale farming (good covering of hills, early haulm destruction, rejection of infested tubers before storage). Cultural practices may at present be sufficient to reduce the effect of tuber damage; these can be taught through outreach activities. With improved technology in the future potato production for the consumer will require greater attention to tuber problems. The question of whether or not tuber and foliage resistance is correlated is perhaps the most important.

There has been recent concern that high levels of teratogenic and gastroenteric compounds in tubers is correlated with high resistance to late blight, but this concern is now lessening as new data is generated. CIP staff should keep informed about work on teratogenic and other substances of suspected toxicity to humans that may be found in blighted tubers. Since it is quite evident that high levels of glycoalkaloid compound in tubers will cause food poisoning symptoms selections and varieties used as parents in a breeding program should be assayed for these compounds.

### Proposed Program

Efforts should be made to initiate a long term project of international scope to determine the "stability" of field resistance. Various approaches may

be needed to recognize shifts in pathogenicity (aggressiveness) and if it occurs, explanations for it must be found.

Two standard differential host sets should be established, one for field resistance and another for the R-genes, and virus-free tubers of these should be produced for international distribution.

The criteria for selection of the set for field resistance should be:

- 1) R-genes should be avoided as much as possible;
- 2) The range in level of resistance should coincide with the 1 to 5 grades of the rating system in Mexico and also include supplemental entities to represent the temperate region response, so as to be able to interpret the results of work in these regions to the advantage of the developing world;
- 3) Include different maturity levels (this may be covered under the previous criterion);
- 4) Incorporate stem blight considerations; and,
- 5) Incorporate different combinations of components of field resistance when identified.

The criteria for selection of a set for R-genes should be:

- 1) Avoid field resistance as much as possible. The series at Pentlandfield ( $R_1$  to  $R_4$ ) has been agreed upon as an initial base.
- 2) Use such single gene genotypes ( $R_1 - R_{11}$ ) and combinations of these genes that are available.

Resistance selection based on tuber tests should be initiated on the clonal collections at both Peru and Mexico. Emphasis should be placed on determining the degree of correlation between tuber and foliage resistance.

For the recognition of pathogenic shift the following studies may be made:

- 1) Continue the unsprayed and sprayed plots with the resistant clonal collection and record tuber yield differences;
- 2) Expand this program to other locations;
- 3) Make objective measurements of the disease and changes in aggressiveness of the fungus; and
- 4) Check and analyze old Toluca data.

To study "weak" links in the complex of field resistance:

- 1) Inheritance studies (the study of inheritance of components of field resistance has been contracted by CIP with the Swedish Seed Association, Svalov);
- 2) Influence of R-genes;
- 3) Influence of environment, temperature, light, rainfall, humidity, nutrition, etc.

The following studies on mechanisms of resistance are needed:

- 1) Resistance to entrance by the pathogen;
- 2) Resistance to invasion; and
- 3) Relation between tuber resistance and leaf resistance. Parts 1 and 2 are included in the Svalov linkage contract.

### III. SOURCES OF RESISTANCE

#### Status of Knowledge

The species Solanum demissum has served as the major source of resistance currently being used throughout the world in programs of breeding for resistance to late blight. Though originally used as a source of a series of independently acting, dominant R-genes, the species has yielded an even more important series of multiple genes which act to give increasing levels of field resistance to all strains that have evolved to infect R-gene-resistant plants. The highest level of this quantitative resistance approaches that given by R-genes to incompatible races though no plant with this type of resistance escapes the disease, particularly in the Toluca Valley of Mexico. The largest collection of clones with field resistance is maintained in Mexico (1300+).

Current and past work indicates that many of the Solanum species other than S. demissum carry high levels of field resistance. Some species such as S. bulbocastanum are not yet available for use because of inability to transfer the resistance genes through crosses to S. tuberosum, is being studied intensively through a CIP linkage contract with Cornell University, as not only a source of new blight resistance genes but also as a source for other factors such as higher yields and better quality. Solanum phureja also offers a potential additional source of blight resistance as do many of the other tuber bearing species. Though the level of field resistance in the currently large collection of S. demissum-derived clones is very high and offers much immediate hope for control of late blight throughout the developing world, it seems only wise that the additional sources of resistance in the various Solanum species should be incorporated into the collection of resistant germ plasm.

In regard to the demissum-based collection of CIP in Mexico it is apparent that it is too large for practical handling. The pedigrees of the clones should be examined and those having the same sources of resistance and less desirable tuber qualities should be discarded on their characters stored in botanical seed. In addition, those selections with highest resistance and best tuber qualities should continue to be mass crossed to provide a true seed source of wide genetic diversity for use in programs of developing countries and to preserve the genetic traits for future use. Special controlled crosses can and should be made for special problems when requested.

Current information indicates that levels of resistance rated 3 or better (based on the Toluca system of scoring) will be needed to reduce the loss from late blight in developing countries. The 3 level is only moderately satisfactory and levels of 2 or 1 would be much more effective. However, at present the best commercial types of resistant clones mostly have only the 3 level. There seems

to be no genetic problem of increasing the level of resistance and maintaining good tuber yields and quality, for such types are now present in some breeding programs.

When a country has the facilities and personnel ready to begin a potato improvement program, the program can move much more rapidly if that country can easily receive vegetative tubers as the source of resistance. If this can be done the primary source of resistance would be selected clones from the Toluca collection, but, in addition, clones with commercial qualities and high field resistance should be imported from national breeding programs of other countries. In time, as the sources of resistance from other species become available in clonal stage, they too can be introduced if needed.

If there is difficulty in clonal introduction then botanical seed of crosses with these clones can be received. Selections for resistance and commercial qualities can be made from these families. Currently there is some danger of introducing the spindle tuber viroid in true seed and precautions to prevent this will be needed.

As resistant material is selected for introduction, insofar as possible resistance to other diseases of importance to a country should be present along with blight resistance. Though of major importance in most developing countries, blight resistance alone will not solve their potato disease problems.

There is conflicting evidence today as to whether or not tuber resistance is combined with field resistance in the foliage and additional studies of this association are in order. Though reports indicate that tuber rot is less important than foliage blight in most developing countries, the source of foliage resistance introduced should also carry tuber resistance when possible. This gives more security of a tuber crop being present under the resistant vines.

Some discussion is being held as to whether or not field resistant sources void of R-genes should be distributed to a country. At present R-genes are scattered throughout the demissum-derived sources of field resistance, and it would be difficult to avoid them. Also, there is lingering doubt among some breeders that if the R-gene type of resistance is completely removed the level of field resistance would be lessened. As the CIP program progresses, attempts can be made to determine whether the very high levels of field resistance exist in clones without R-genes and these used to develop material for distribution.

### Proposed Program

Maintain, in reduced numbers, clones with the genetic sources of resistance now in the Toluca Valley collection - mostly S. demissum sources.

Incorporate sources of resistance of other species into the germ plasm base now in Toluca, and transfer these sources into breeding material easily used by leaders of projects in developing countries.

Prepare quantities of true seed for distribution, both through mass pollination procedures and controlled crosses.

Arrange to obtain the best blight resistance in good tuber clones of breeding programs in other countries for inclusion in the CIP collection at Toluca, or arrange with such programs to introduce them directly into the developing country.

Combine resistance to blight with resistance to other diseases in the same clones.

Begin to collect information on the level of tuber resistance in the Toluca collection.

Begin to develop, if possible, highly field resistant material void of R-genes.

#### IV. BREEDING METHODS

##### Status of Knowledge

Methods of breeding for resistance to late blight currently in use by the leading potato breeding programs of the world have succeeded in producing good or adequate levels of field resistance together with the added temporary additional major "R" gene protection. These types of resistance have been primarily derived from Solanum demissum, and they cannot readily be separated. Thus, most breeders have continued to use both types although the ideal is to develop high levels of field resistance alone, but it is not yet known if high enough levels are feasible for most situations. The determination of field resistance in the presence of major genes is done at Toluca, Mexico, the best known place for this because of the high incidence of the disease and the presence of the two compatibility types that are needed for the sexual stage of the pathogen, which presumably permits the ready formation of many races and their preservation in the field in the form of oospores.

Additional sources of resistance are known, and especially interesting is Solanum andigenum which appears to have an entirely different set of genes that can be easily incorporated into commercial type cultivars.

Clones with resistance and high quality that exist in some programs may be useful elsewhere. Selection from progeny of crosses between some of these may readily yield the needed clones which can, by rapid multiplication techniques, become widely grown in a 5-year period. An adaptability problem may be day length sensitivity.

There is a need for a standard rating system to evaluate late blight that could be readily used. It should therefore be pictorial and simple to interpret.

### Proposed Program

Different sources of resistance should be combined.

Resistance sources should be evaluated for as many characteristics as possible so as to make the most suitable clones available according to the needs of each location.

Seed of superior crosses should be made widely available.

The capacity of national programs to receive these materials must be improved or developed when the need exists.

Late blight tests must be conducted first at a national or regional level. Regional testing locations should be promoted.

Results of national or regional tests should be compared with subsequent tests at Toluca to assess their relative value.

A standardized rating system for late blight evaluation should be prepared that incorporates visual aids, but that essentially follows the system now in use at Toluca.

Evaluations at Toluca by CIP personnel should be at approximately 8-day intervals from the inception of blight on the standard susceptible (Alpha) until it is dead. Data for both the susceptible and resistant (Atzimba) standards will continue to be included. Meteorological information should be included in the reports.

Yield information should not be routinely taken, but may be supplied upon special request for multiple tuber clone entries.

Additional help to breeders using the Toluca test should be given when possible by making the crosses or selfs they may request, using detached

stems in the greenhouse. Open pollinated seed may also be saved. When crosses with "quality parents" are desired, these may be sent and should be sprayed for blight control prior to crossing.

Since breeder's rights may pose problems with the distribution of the better entries in the Toluca test, it is recommended that breeders should be given two alternatives: 1) Sign an agreement that when they submit clones for testing that these are of a category that is available to anyone qualified and interested; 2) Submit clones that will be unavailable to others but forsake the possibility of being a recipient of clones from other sources in the program. It is further suggested that CIP consider making it a requirement that all materials be shared, to the benefit of breeders throughout the world, especially in the developing world. All materials must be considered free for use in crosses. Note: CIP policy on this issue has been defined as follows. Since the funds CIP receives are specifically designated for providing assistance to the developing world, all testing done at CIP expense will require that the interested party sign an agreement granting CIP permission to use the materials tested in its program to help the developing countries of the World. The naming of varieties is not a CIP objective, so that no tested clone could be selected for varietal release by CIP (15 October 1973, E.R.F.).

## V. THE FUNGUS

### Status of Knowledge

The most important characteristic of P. infestans having a bearing on the development of resistant varieties is its extreme variability. The literature documents well the story of the rapid appearance of new pathogenic races which render the R-gene resistance ineffective. The question now of most concern is whether or not the plastic character of the fungus will allow it to overcome the resistance controlled by the series of multiple genes providing the quantitative resistance commonly referred to as field resistance.

The principal modes of variation recognized today are mutation followed by selection; and sexual recombination. The latter as a factor in nature is known only in Mexico. The planning group recognized that the sexual stage is not necessary and probably has little effect on the development of R-gene-specific pathogenic races because of many records of rapid collapse of all known R-gene resistance in regions of severe blight development where the sexual stage does not exist. The rapid development of pathogenic races through asexual variation is usually attributed to random mutation. Some evidence exists that asexual pairing may occur leading to recombinants and



possibly different races. Also, some thought is being given again to the hypothesis of host-induced variation:

The stem infections of young plants at the soil level, the infections of leaves touching the soil, and the recovery of the fungus from soil in which potatoes were not grown in the previous two years suggest that oospores play an important role in the survival of the fungus in Mexico. The presence of oospores in the soil at plant emergence may provide an immediate inoculum with the full range of pathogenic races from recombination of the race characters present in the population. The process of evolving complex races through the sexual stage would be short-circuited. If there is slower evolution to increased aggressiveness to overcome field resistance, the sexual stage would store that level of aggressiveness for continuing the upward change in aggressiveness during the next cropping season:

Mexico studies have also shown that both the  $A^1$  and  $A^2$  compatibility types occur in all sections of the country so far surveyed. At present the  $A^2$  type has not been found outside of Mexico. Thorough search for the  $A^2$  type has not been made in other Central and South American countries but should be done because of the reasons noted in the above paragraph. Meanwhile, extreme care in movement of potato parts from without Mexico should be continued.

Race surveys of the past have indicated that the population of races in a country or region may be influenced by the R-gene make-up of the potato varieties being grown. Thus it is believed that detailed race surveys in new countries would serve no useful purpose and that the information gained through use of the R-gene differential hosts would be sufficient. As breeding incorporates whole new pools of genes for resistance into the genetic material being distributed it is possible that studies of race development will need to receive attention. Further it is believed that maintenance of an extensive set of physiologic races for identifying the R-genes in a potential new variety would not now be a function of the Center.

#### Proposed Program

Stimulate studies to determine whether or not the fungus will increase in aggressiveness.

Encourage the continuation of the studies now going on in Mexico on the role of the sexual stage in survival and the epidemiology of disease development.

Survey other countries of the world especially Central and South America for the occurrence of the  $A^2$  compatibility type.

As new gene pools from other species are included in the Center's collection, begin observations for the occurrence of additional sets of pathogenic races to correspond with the resistance genes of the new pool.

Should it become evident through some of the suggested future research, that some major genes (R-genes) are not overcome in the Toluca test, consideration should be given to the value of maintaining a collection of specific races to overcome these R-genes and using them to inoculate spreader rows in the field at testing time.

## VI. ADAPTATION TO DIFFERENT ENVIRONMENTAL CONDITIONS

### Status of Knowledge

The expression of field resistance varies with numerous factors such as the age of the potato plant, the age of the leaf (or its position in the plant), moisture factors (rainfall, relative humidity, soil moisture), temperature, source of origin of the plant, planting density, nutrition, daylength, light intensity, and the presence of viruses (recently shown to reduce late blight incidence).

Considering some of these factors, there is concern that some clones are more susceptible under the short day conditions of the late blight test at Toluca when they are of long day origin. This greater susceptibility is in the form of larger lesions, which may reflect the shorter life span induced in the plant. Day length and temperature factors are considered to interact. In general, temperatures favorable for the potato, are also favorable for late blight. High light intensity appears to increase the resistance of S. tuberosum.

The factors that control field resistance interact with the environment, hence field resistance may vary with each set of conditions.

### Proposed Program

Coordinate observations of late blight incidence on the differential sets at locations differing in day length.

Observe the range of adaptability of Phytophthora infestans in the studies of adaptation of the potato to more extreme temperature conditions (frost resistance, adaptation to the lowland tropics). Differential host sets should be included.

Study the effect of varying conditions during the late blight test, e.g. nutrition, plant density.

Daylength insensitivity should be sought and used.

Other methods of controlling late blight should be studied and applied, such as cultural practices (high ridging for tuber rot control) and other methods of control such as breaking the inoculum availability cycle. The progress in chemical control should be monitored, particularly the research and development activities with systemic fungicides.

## VII. ASSISTANCE TO NATIONAL POTATO IMPROVEMENT PROGRAMS

### Proposed Program

Make available resistant materials. The best clones in terms of resistance and quality amongst those in the Toluca collection are available for immediate shipment to requesting programs. Usually about 10 clones are sent, and 10-15 requests are handled per year. These clones may be of adequate commercial level for some situations, thus requiring only multiplication. Shipments should be made in accordance with the regulations of the recipient countries. Tuber desinfestation by chemical treatment is the normal practice, and these materials are free of detectable viruses. When quarantine regulations make introductions to a country difficult, the utilization of regional centers may be useful.

In addition to using the clones of the CIP collection an effort should be made to locate varieties from sophisticated programs that may be useful to others.

For future distributions, consideration should be given to the selection of clones with very high field resistance and no major genes, and to combining different sources of resistance. These should be crossed with clones for desirable characteristics for particular locations (resistance to other diseases, skin and flesh color) or generally desirable characters (high dry matter, high protein, high yield). These materials could be distributed as botanical seed to locations that can screen the segregating populations, or could be grown out and screened for one or more characters and the tubers sent to locations that can utilize them. The blight differentials should be included at such locations.

Develop the capacity to receive materials. Locations that lack adequate programs should be helped in the task of establishing them or

improving the ones they have. Present or future potato program members should be trained at CIP or other specialized potato training schools and in the field as a part of CIP's outreach function. It is essential that programs have a designated leader and a defined structure to be effective. Adequate facilities must also exist or be developed.

Seed Programs. Even the best varieties have little impact unless an adequate seed multiplication program exists that maintains the good health of these and makes them available to farmers. The training of seed specialists and the promotion of good seed programs is essential.

Interaction with other programs. The Center should provide guidance to bilateral aid programs to ensure they are best equipped to succeed, and should try to utilize the progress made by successful breeding programs to help others, utilizing the International Late Blight Test as the avenue of approach.

## VIII. PROPOSED PROGRAM

### Outreach and Development

The outreach function of CIP is to make available the late blight resistance sources in existence and to help national programs integrate these into their improvement programs.

Resistant clones available in the Toluca collection will be made available for shipment to national programs that want to test these sources of resistance. Import regulations of the recipient countries must be observed. Tuber desinfestation by chemical treatment will be practiced, and these materials will be free of detectable viruses. Regional centers will be established and introductions of suitable clones through these will resolve some of the quarantine regulation problems that exist. Clonal materials will permit a breeding project to get underway rapidly. True seed will also be available, and an increasingly wider source of resistance genes will become available (see Maintenance and Development of Resistance Sources).

The development of national programs to evaluate and utilize materials, and to improve their capacity to do so, are CIP goals. There are many mechanisms by which CIP can train staff members of national programs so that they can more effectively receive late blight resistant materials. These programs must also have adequate facilities.

Seed programs capable of adequately multiplying and maintaining new resistant varieties free of other diseases are essential. CIP conducts seed production training programs at both its Peruvian and Mexican facilities.

#### Testing for Blight Resistance at Toluca

The international late blight testing program, that was conducted under auspices of the Rockefeller Foundation during many years, will continue to be carried out by CIP for as long as it provides a service to plant breeders that cannot be fulfilled effectively by more simple, less costly means. The Toluca valley offers the most stringent blight test known in the World, and will be available for final evaluations of selections made by screening at national (and when possible regional) sites. Regional testing sites will be promoted by CIP, and their relative value assessed by comparison with Toluca.

To improve the test, a standardized rating system for late blight evaluation will be developed (or an existing one selected) that incorporates visual aids and essentially follows the system so far in use at Toluca. Evaluations will be made at approximately weekly intervals from the inception of blight on the standard susceptible (Alpha) until about 2 weeks after it is killed and data on this standard and the resistant standard (Atzimba) will be reported. Also meteorological information for the period will be included. Yield information will not be routinely taken, but may be requested for multiple tuber clone submissions. Additional help to breeders, such as the execution of requested selfing and crossing, will be provided when possible.

Since CIP's objective is to assist the nations of the developing World, all testing done will be upon the condition that the materials tested can be used by CIP for that purpose.

#### Maintenance and Development of Resistance Sources

The large collection of resistant clones in the Toluca collection will be reduced in number - these contain mostly S. demissum resistance genes. The best clones developed by breeding programs around the world will be added to this collection. Any new sources of resistance found in CIP programs in Peru will be included in the collection; those developed by others will be requested. This collection will be evaluated for as many

characteristics as possible so as to be able to make the most suitable clones available according to the needs of each location.

Various combinations of resistance and other desirable genes will be developed. Resistance genes from species not presently available in the Toluca collection will be incorporated into more desirable clones more readily usable in breeding programs. Different sources of resistance, including tuber resistance, will be combined. Daylength insensitivity genes will be sought and incorporated into desirable resistance sources. True seed of superior crosses, from both mass pollination and controlled cross methods, will be made available. Desirable combinations of resistance to late blight and other diseases or factors will be produced. The glyco-alkaloid content will be determined in order to avoid clones with high levels for use in breeding programs.

In Peru an attempt will be made to locate highly resistant clones with no major genes, which would reduce the need for testing at a location such as Toluca, and might permit breeding and selection work to be done entirely wherever late blight occurs.

#### Monitoring Pathogenicity

To assess the pathogenic potential of the late blight organism (Phytophthora infestans), two standard differential sets will be established, maintained virus-free and distributed: one for field resistance (multigenic resistance) and another for the major R-genes (monogenic resistance).

The field resistance differential clone set will consist of 5 clones representing the five grades of the rating scale among clones adapted to the short daylength conditions of Toluca (selected amongst many clones whose ratings have been previously recorded and which will be carefully scored during two growing seasons, and also tested to Race 0 and submitted to qualitative and quantitative inoculation tests) and a similar long daylength set will be selected by North-European scientists (possibly Swedish, Dutch and Scottish).

The major gene differential clone set will consist of the series at Pentlandfield comprising the 12 single gene genotypes r, and R<sub>1</sub> through R<sub>11</sub> plus additional combinations of these genes that are available. CIP is requesting the Scottish Plant Breeding Station to assume the responsibility of maintaining both clone sets and distributing them on CIP's behalf to interested programs around the world.

Since so much responsibility for the success of the Center's programs depends on the multigenically inherited resistance, it is important to know beyond a doubt whether the fungus can also adapt to this type of resistance as it has the monogenic resistance. The accumulated data on blight incidence in fungicide sprayed vs. non-sprayed plots at Toluca will be analyzed to determine if it shows that a shift in aggressiveness of the fungus has taken place. This research will be continued utilizing the more precise rating system that will be developed. The field resistance clone set will be included in future tests, and will be rated in other locations around the world for the same purpose. Studies on the repeated passage of an isolate through clones with different levels of field resistance will be encouraged among collaborators or CIP's own staff.

The role of the sexual stage in survival and the epidemiology of disease development will hopefully continue to be studied by the pioneering Mexican scientists, or by visiting scientists with CIP at Toluca. The presence of the compatibility type A<sup>2</sup> will be investigated in Peru, and surveying for it in other South American countries and Central America will be encouraged.

#### Field Resistance

Studies on the mechanism of field resistance are being conducted through a CIP linkage project at the Swedish Seed Association in Svalov, Sweden, under the direction of Dr. Vilhelm Umaerus. Emphasis is being placed on 1) resistance to entrance by the fungus into the leaf, 2) resistance to growth of the fungus in the leaf, and 3) a reliable method for assessing the possible correlation between leaf resistance and tuber resistance. Similarly, studies on the inheritance of the components of field resistance are being initiated at Svalov.

Additional studies on tuber resistance would be desirable. If there is a correlation between foliage resistance and tuber resistance a method of foliage assessment might be developed which reveals the correlation. This could accelerate breeding programs concerned with tuber resistance. Research on the nature of field resistance in the tuber may be needed to bring this about.

#### Late blight control

Research to control late blight by other means than resistance will be considered, such as the value of high ridges for tuber rot control. The

progress in chemical control will be monitored, with special interest on the research and development of systemic fungicides.

#### Adaptation of the potato in relation to blight

The incidence and significance of late blight will be an integral part of CIP research programs on the adaptation of the potato to environments beyond its recognized normal range (e.g., lowland tropics). The differential host sets will be included at these research sites. Information will be requested from collaborators receiving these sets so as to accumulate information on their response to differing daylength.

#### IX. ADDITIONAL COMMENTS

To stimulate the discussion I formulated a few questions, which were sent to Dr. BLACK and Dr. GALLEGLY. These are the questions and the answers:

a) Both of you have supervised in the potato work in East Africa (Kenya and Uganda), and interesting presentations of this work were given at the meeting in Lima last year with particular emphasis on late blight resistance. Do you find the present germ plasm and the level of resistance it contributes sufficient for future breeding in East Africa? If not, should CIP give priority to search for field resistance to blight in the germ plasm available to the center?

BLACK: "We have a wide range of breeding material in Kenya and we can produce group I and group II resisters in reasonable quantity. We are releasing a new variety at the moment which reacts somewhere between groups I and II. It looks very promising in every way, e.g. yield, size, shape and cooking quality. It has not yet been tested in Mexico, but the screening results of progenies bred from it, indicate field resistance without the interference of R-genes. Nevertheless, I would suggest that CIP should examine the material they have and try to improve upon what is already available."

GALLEGLY: "Dr. WURSTER and I found that the levels of multi-genic resistance present in our breeding program gave excellent performance in Uganda. The same was true for this type of resistance in clones from the programs of Mexico, Netherlands, Scotland, etc., tried in Uganda."



Thus there is sufficient resistance to significantly contribute to the East African programs. I personally believe that this type of resistance will be stable in most areas where late blight is a factor. The major job to be done is the one of selecting resistant clones adapted to the particular geographic area which give high yields and have good quality. Meanwhile the Center could well benefit by identifying commercial-type clones with resistance as well as accessions of Solanum species with resistance for trials in a country to begin the selection process. The first approach in a new program would be to start with named varieties and clones approaching variety status. If needed then one could turn to the species."

b) Do R-genes still contribute to the value of blight resistance or do they create more problems in screening and evaluation of field resistance than merits their existence?

BLACK: "Since R-genes are commonly found in selections bred from S. demissum, it could be difficult to get rid of them without losing good breeding material. Their presence could protect a variety completely for some years and if that variety is known to be moderately field resistant, the advent of new races should not be very serious. In other words, field resistance must be present, and if effective R-genes are also present they would give added protection for a time."

c) What is your experience of the adaptation of field resistant clones to the particular environmental conditions in East Africa? A strong influence of daylength is often mentioned with a lower level of field resistance under short day. I learned from WURSTER that blight comes very early in Uganda. What effect has that on breeding for resistance?

BLACK: "I find little difficulty in producing field resistant clones adapted to short day conditions and higher temperatures. Types that are too late in maturing for Europe seem to give best results. We are trying some in the lowland tropics to see if they are also tolerant to high temperatures."

GALLEGLY: "There is strong circumstantial evidence that the expression of multigenic resistance is influenced by day-length and light intensity. I believe that day-length effects are really those effects that influence maturity, and the resulting influence of increasing maturity on reducing the expression of resistance. Low light intensity also will reduce the level of resistance as indicated in greenhouse studies, and all of us in certain areas of the United States have seen multigenic resistant plants with a lot of blight following a few weeks of constant cloudy, rainy weather. During the rain periods in Mexico and Uganda I observed as a usual condition the clouds, the rain, and bright sunshine in between geographic areas. Thus, a breeding program is most effective when selections for day-length

adaptability and resistance are made under the local conditions of the potato growing regions of a country.

In some of our work cooperatively with Niederhauser in Mexico we have identified a few clones that are resistant in Mexico and susceptible in Maine and West Virginia. The reverse is the usual case. When a clone is resistant in the Toluca Valley in Mexico it usually is also resistant in Northern U.S. The few exceptions probably are interrelated with day-length and maturation. "

d) The Toluca Valley, Mexico, has been a valuable testing ground for breeders from many countries. Do you have need for this testing ground for future work?

BLACK: "R-genes can be a nuisance in screening for field resistance if the appropriate races are not available. They can completely mask the degree of field resistance present by giving a hypersensitive reaction. When such plants are tested in the Toluca Valley there seems to be every chance that the necessary races will be present to give the true picture. For that reason, I think the trials in the Toluca Valley should continue. "

GALLEGLY: "Toluca Valley is probably the best site in the world for a 'late blight proving ground. " The advantages are numerous. Diversification of tuber-bearing species and concept of the "home of the potato", whether it is or not, applies to this site. The fungus has been in the presence of diverse host germ plasm from the beginning and thus should be developed to its highest order of pathogenicity. The day-length characteristics are closer to those of most tropical areas yet experience has told us that most selections with resistance are even more resistant in northern hemisphere areas (I do not know about performance in the southern hemisphere). Experienced staff and adequate facilities have been developed over a period of years at this site. "

e) Have you information on the accordance between blight readings in Mexico and other locations?

BLACK: "I have some clones that have been tested in Scotland, Mexico and Kenya and the results are more or less comparable. In general, the higher temperature in the tropics seem to favour the fungus and give slightly larger lesions, but the difference is not big enough to cause concern. As Dr. WURSTER said, blight can come early and kill a susceptible crop before it has bulked, but even a group III resister would yield a crop in the same conditions - although it would benefit from spraying with fungicide. "

GALLEGLY: See c) and d) above.

f) CIP has a rather intensive parental breeding program on bacterial wilt resistance. Do you find it feasible to have a similar program for late blight?

BLACK: "Resistance to bacterial wilt is a much more difficult problem than resistance to blight because good, highly resistant clones are not available and a simple, reliable, method of screening progenies has not been devised. By comparison, the blight problem could be regarded as more or less under control."

GALLEGLY: "I believe I have essentially suggested this approach above for a Center program. This may not be a parental development program per se but the identification of clones and accessions with resistance which a country could secure for their first-step trials. Some of these may be named varieties, and from the collection it may be possible to quickly relieve the situation of that country. There is the problem of introduction of vegetative clones through the plant quarantine facilities of a country which must be overcome. Either disease free clones or true-seed lines may be needed. CIP might later do the actual crossing using parents identified from the first-step trials as having qualities approaching those desired in a country. The true seed could be sent to that country relatively easy."

g) Dr. GALLEGLY, you have devoted much work into the field of genetics and cytology of P. infestans. Do you find this to be within the scope of CIP's program? If so, which particular questions would you recommend CIP to consider?

GALLEGLY: "An understanding of the genetics of host-parasite interactions in the late blight disease and the cytology, particularly the nuclear situation, is important for persons working directly with the Center's programs. I question whether the Center should emphasize this aspect of research on late blight, particularly the cytogenetic area. If the need is felt, perhaps the Center could contract for such studies somewhere in the world where an unbiased approach could be made. There may be some need for direct Center research on furthering our knowledge of the genetics of the disease, and this need might be felt as higher and higher levels of multigenic resistance are introduced into commercial culture. Will this resistance continue to hold in all geographic areas? It is now clear that none of the monogenic factors will hold up in the face of this pathogen, and there is really no good evidence that these qualitative genes serve in an additive manner as seems to be the case with the other quantitative genes which appear to be completely independent. The use of the words "seems to be" and "appear to be" indicates need for generating additional information about the relationship of the two gene systems. Another problem facing us today is whether or not the multigenic resistance is associated with high levels of undesirable edible qualities of tubers as-

suggested by the hypothesis papers of RENWICK and associates. I think not but the question must be answered. We got into trouble with most of our highly multigenic resistant lines because of very high levels of total glycoalkaloids - much higher than in the variety Lenape which was removed from our marketing channels because of it. Fortunately a few lines were low in TGA & still resistant. What about other chemicals, such as rishitin and chlorogenic acids? Are these compounds teratogenic, and are there other teratogens associated with resistance? The Center would not want to be guilty of improving the quantity and nutritive value of the potato over the world only to find an increase in birth defects as a result."

On my request Dr. GALLEGLY answered other questions of importance:

"I believe that we have reached a plateau of multigenic resistance to late blight, and the real and big job now before the Center is to apply this knowledge in development programs where blight is a factor. This will be a slow process and require a lot of money and energy. Not only will we have to identify sources of resistance in that region, it will have to be incorporated with resistance to other diseases and other desirable horticultural characters. Further, not just one variety will do the job - chip, bakers, etc., and early, medium and late varieties. There is a long road ahead in reaching this goal. Meanwhile we should be looking for a higher plateau of resistance and a higher plateau of yielding ability for use much further down the road.

Another more specific study of shorter range might be on correlations of tuber and foliage resistance with the idea of shortening our screening procedures."

Dr. KENNETH SAYRE is at the moment on a Rockefeller post doctoral assignment with the International Potato Program in Mexico and has recently submitted to CIP a list of recommendations for future activities of the CIP regional program in Mexico. Dr. SAYRE prefaces his list of recommendations by stating that "they do not call for additional funding or personnel, but provide for a more efficient use of the funds and personnel that are currently available." These are excerpts of Dr. SAYRE's recommendations concerning the late blight work:"

a) The International Late Blight Test. Perhaps the most important project that is conducted in Mexico is the International Late Blight Test. Each year several cooperating potato improvement institutions send potato germplasm in tuber form to screen for late blight resistance in the Toluca Valley. The tubers are planted in a quarantined area at the CIMMYT station in the Toluca Valley and regular blight readings are taken. These readings

are sent back to the cooperators to aid them in the development of blight resistant varieties. This is the extent of the contribution that is made by the Mexican program. I suggest that more can be accomplished in Mexico to further assist the cooperators.

First, if a cooperator would specify that crosses and/or selfs should be made using the most resistant clones present in his material, this could be accomplished in Mexico using cut-stems. The best resistors can be identified in the field early enough to allow cut-stems to be harvested. A greenhouse is available to make the crosses and I intend to use the balance of money I have left in my Rockefeller grant to install an airconditioning and humidifying system in the greenhouse to provide more optimum conditions for making crosses using cut-stems. The personnel now working in the program are well versed in crossing techniques. The seed resulting from the crosses or selfs would be returned to the cooperator and should greatly increase the rate of advance of his selection program.

Second, if a particular cooperator has blight susceptible clones that he would like to cross with the most resistant clones in the material he sends, these clones could be planted in a plot that would receive fungicide and the crosses could be made. In this way he will have the hybrid seed at least 6 months earlier than if he had to wait until he received the blight readings from the material he submitted for testing.

Third, the possibility also exists for cooperators to send populations of botanical seed for blight testing. This seed could be planted in the greenhouse during the winter and the seedling tubers would be ready for planting in the field by May for blight screening. Again crosses or selfs, at the direction of cooperators, would be made and the resulting seed returned for selection in the home country. This cycle could be continued until a population is developed with a high level of blight resistance and with maturity, tuber characteristics, and other disease and insect resistances selected for in the home country. I estimate that by staggering the planting of botanical seed in the available greenhouse, approximately 7000 seedlings could be handled each year. In addition, the exchange of botanical seed between the cooperators and Mexico would minimize the quarantine problems in Mexico.

Fourth, many cooperators, who send material to Mexico for late blight screening, use preliminary greenhouse or growth chamber screenings to eliminate a portion of the material undergoing selection. I propose that the late blight test in Toluca could be used to evaluate the effectiveness of these preliminary screenings. The cooperator could conduct his screening as usual but, instead of sending only his selected clones to Mexico for testing, he could send all clones that were involved in the screening process. The proportions of the clones that possess

resistance at Toluca in the unselected and selected populations would provide an indication of the efficiency of the screening test.

These four recommendations that I have set forth concerning the conduct of the International Late Blight Test in Mexico represent by no means all of the possible added contributions that could be undertaken. I hope that the late blight workshop scheduled for August in Mexico can provide a forum for the discussion of these and other possibilities.

b) Maintenance of the Germ Plasm Bank of Late Blight Resistant Clones. There are approximately 1000 clones with varying degrees of resistance to late blight that are currently being maintained by the Mexican program. Of these 1000, approximately 300-400 have an acceptable level of late blight resistance. Some of the other clones have other desirable characteristics and, therefore, should also be maintained. The rest are still present in the bank because of the natural reluctance to discard unique clones.

Last year, I initiated a collection of open-pollinated botanical seed from all clones. We collected seed from about 80% of the clones. This year, all clones were planted and seed will again be collected. Therefore, following this year, I recommend the elimination of those clones that have poor late blight resistance; that do not possess other notable, beneficial characteristics; and for which we have successfully collected botanical seed. The cost of their maintenance does not justify their existence especially since their genetic potential is available as botanical seed. I would like to see the number of clones in the bank reduced to between 600-700 clones. I would, however, like to receive advice from Dr. ROWE concerning this situation. We should look upon this germplasm bank from two viewpoints; 1) as a reservoir of late blight resistance; and 2) as a broad-based population of primarily *Tuberosum* germplasm. This second viewpoint should have some bearing on whether the decision is made to eliminate certain clones.

I am in complete agreement with the manner in which the germ plasm bank is planted each year. That is, 10 tubers of each clone are planted and sprayed with fungicide and insecticide, and 10 tubers are planted and sprayed with only insecticide. This allows a continuous monitoring, over years, of the stability of the resistance of each clone by comparing the fungicide versus no fungicide plots, and the late blight readings from year to year. This data, however, needs to be pulled together and summarized, and kept current.

I would like to see a more conscious effort expended towards the dispersal of the genetic potential of the bank. Until last year, only

tubers could be distributed (which involved quarantine limitations in many other countries). Now we can also begin to distribute botanical seed. This should be more fully publicized for the benefit of potato breeders around the world.

CIP has contracted a linkage program to Svalov, Sweden, which commenced July 1st this year. The overall objective of this program is to assist the International Potato Centers in the background work needed for the development of late blight resistance in the national potato breeding programs of the developing countries. Five major goals have been recognized and the procedure to assist the Center in this work is outlined in a proposal which has been accepted by CIP. The five goals are as follows:

1. To cooperate in studies of the germ plasm available to the International Potato Center in search for sources of field resistance.
2. To conduct studies on the mechanism of resistance with emphasis on:
  - a) resistance to entrance of the parasite into the leaf;
  - b) resistance to growth of the parasite in the leaf (lesion development);
  - c) relation between leaf resistance and tuber resistance and the influence on other tuber characters, e. g. cooking quality.
3. To conduct genetic studies, concerning the inheritance of components of field resistance.
4. To cooperate in studies of the adaptation of field resistant clones to temperate, subtropical and tropical conditions with emphasis on the expression of resistance.
5. To extract information from the above mentioned goals with influence on development of methods of selection, evaluation and other aspects of breeding potatoes resistant to late blight.

APPENDIX I

Position Paper for the

Late Blight Project Planning Conference of CIP

Vilhelm Umaerus

This paper has been prepared on request by CIP to serve as a background for discussions during the late blight planning conference in Mexico City, August 22-27, 1973. It is not my intention to present a complete and up to date review of the present status of research concerning the late blight organism and its host, merely to mention those aspects I feel relevant for the discussions and to give a framework for what should be discussed.

Although references are mentioned not all statements or informations are supported by producing names of authors, neither is there a list of references. I think most of us are familiar with the references mentioned. The term field resistance is used in the way potato people have communicated so far, I think there is no danger of misunderstanding although horizontal resistance may be the more legitimate term.

During the course of the work questions have arisen and I have forwarded those to some of the participants of the meeting. I think the answers are of great interest for the discussions. Therefore they are presented in extent at the end of the paper. I am most obliged to Dr. WILLIAM BLACK and Dr. MANNON E. GALLEGLY for their replies.



### Introduction and Justification

The potato late blight fungus, *Phytophthora infestans*, is known as the causal agent of one of the major diseases of the potato crop. The fungus probably originates from Mexico and its surrounding countries; now the distribution of the fungus is for all practical purposes coincident with that of the potato itself.

A vast expense of money and labour goes each year on control measures - blight forecasting, spraying with fungicides, haulm killing, production of disease free tubers, breeding of resistant varieties - in those countries, which can afford such measures. Chemical control measures are too expensive for most developing nations especially when the crop is produced by subsistence cultivators. In tropical and subtropical regions blight epidemics are occasionally catastrophic. In some regions, such as Mexico and East Africa, heavy blight attacks may prevent potato cultivation in a number of districts, where it would be possible with the use of resistant potato varieties, adapted to these areas. In other regions the major loss from blight is the loss of the whole of the second half of an otherwise ideal potato growing season. In all of the potato growing regions of South America late blight is a major concern as it is in India and Pakistan.

The breeding of resistant varieties seems to be the only possible way of control of the disease in developing countries, and several breeding programs are now in progress. The world wide interest in the development of late blight resistant varieties is demonstrated by the fact that 29 countries have in the past utilized the field testing facilities in the Toluca Valley of Mexico for determining field resistance to late blight. Those countries are: Argentina, Australia, Bolivia, Brasil, Canada, Colombia, Costa Rica, Chile, Denmark, East Germany, Ecuador, England, France, Guatemala, India, Ireland, Japan, Kenya, Netherlands, New Zealand, Pakistan, Peru, Poland, Scotland, Soviet Union, Sweden, Uganda, United States, West Germany.

Late blight and brown rot (bacterial wilt) are the two major diseases, which must be controlled for adaptation of the potato to the low land tropics. CIP has recently, in a potato bacterial wilt project planning conference, adopted a scheme for developing bacterial wilt resistance, including late blight tests (originally developed by the Wisconsin breeding program).

The breeding of late blight resistant varieties seems to be the only possible way of control of the disease in developing countries and several breeding programs are now in progress. The breeding work has so far met with certain difficulties mostly because of the variability in pathogenicity of the fungus. Race specific resistance, although promising in the beginning, is of limited value especially in areas where the fungus is sexually reproduced, but also in other regions this type of resistance has a short life time due to

mutations and somatic recombinations. Most potato breeders now primarily breed for field resistance with a secondary interest in R-gene resistance.

#### A. The host

##### 1. Resistance in the foliage.

Field resistance. Field resistance has been attributed to such factors as resistance to entrance, and to growth of the parasite manifested in the number of lesions formed, rate of necrosis, rate of advancement of mycelium, intensity of sporulation and generation time. In addition, factors such as the nature of the leaf surface, growth habit of the plant and type of crop canopy produced, which control the persistence of moisture on the leaf surface, may play a role.

Field resistance may thus be regarded as a complex of different factors, the sum of which determines the actual level of resistance to the parasite apparent under field conditions. It is unlikely that determination of any single factor will give an accurate picture of the true level of resistance. On the other hand screening and selection methods will be more adequate and efficient if based on single, well defined, major components of the field resistance complex. A genetic analysis of the inheritance of field resistance requires that the different factors are studied separately.

As a prerequisite for infection of potato leaves, water droplets or films must persist long enough for germination and penetration of the host to take place. Laboratory and field observations have revealed that highly resistant clones develop fewer lesions per plant than others. There is also evidence of a longer minimum inoculation access period in varieties with a low infection frequency.

Little is known of the nature of this effect. The germination of sporangia and zoospores, or the growth of the germ tubes, the penetration, or the establishment of the parasite in the cells of the host may be affected.

When germination and penetration have succeeded the fungus has to establish a food relationship with the host in order to survive. Mycelium grows in the intercellular spaces of the leaf tissue, sending haustoria into adjacent cells, and once a food relationship is established and the microclimatic conditions are favourable, the fungus sporulates, sending sporangiophores through the stomata of the leaf surfaces. The rapidity of this innovation of the leaf varies with the variety. Observations on incubation time, lesion size and growth, generation time, and sporulation capacity are the characteristics commonly used for measuring field resistance.

A food relationship has been associated with the amino acid requirements of *P. infestans*. The apparent shift towards increased susceptibility when tuber production starts has been attributed to an increased protein hydrolysis at the onset of tuber formation, but also to a shift in the total carbohydrate metabolism of the plant.

Several observations indicate the presence in the potato of factors influencing hyphal growth independently of cell necrosis. It is difficult, however, to separate the effects of food supply from the action of defense of the invaded leaf tissue. The injury to host cells by the invading hyphae leads to the accumulation of phenolic oxidation products which initiates browning. Oxidizing enzymes and their substrates have been implicated in the infection process. A correlation between peroxidase activity and field resistance has been found under certain defined conditions. Ageing, or mechanical detaching of leaves, or flaming of the petioles lead to the accumulation of soluble nutrients in the leaves and also to a lowered resistance. The amino acid accumulation and its influence towards higher susceptibility can also be explained on the basis of polyphenol metabolism, since an increase in the soluble nitrogen - phenolics ratio often lowers the toxicity of the polyphenols.

The physiological and biochemical factors, which influence the development of a blight lesion, are partly understood. It may be concluded that measurements of the rate of development or size of both the holonecrotic and plesionecrotic zone of a blight lesion reflects the competition between the rate of advancement of the mycelium and the necrosis of the host. The sporing capacity measured as number of spores produced per lesion, or number of spores produced per unit area of sporing zone, may be a function of those characters.

Influence of non-genetic conditions. Ontogenesis and light regime (day length and light intensity) have a large influence on the phenotype of field resistant varieties. This will be discussed later.

A considerable predisposing effect of mineral nutrition on field resistance has been reported by several authors. High nutrition might under some conditions be associated with increased resistance, probably due to a prolongation of the period of intensive vegetative growth of plants. Susceptibility seen as lesion growth increases at high levels of balanced nutrition, which apparently creates a tissue highly favourable for the growth of the pathogen. The infection frequency is very little influenced by mineral nutrition. Lesion growth and sporulation intensity increase with increased levels of potassium and decreased levels of magnesium. The rate of lesion growth is more strongly influenced by the treatments than the rate of necrosis.

Genetics. Field resistance in hybrid derivatives of the wild species *S. demissum* is controlled by many different genetic factors and inherited in a

polygenic fashion. The complex character of field resistance outlined above represents the combined effect of genetic factors controlling these entities. In the course of hybridization and backcrossing to commercial varieties to improve quality and yield these factors become dispersed with consequent reduction in degree of resistance. A detailed genetic analysis of the components of field resistance has not been made, however, a valuable working tool for genetic studies will be the production of dihaploids.

Stability of field resistance. The main advantage of the use of field resistance is that no sudden breakdown is to be expected. The complex expression of field resistance is a guard against sudden changes in P. infestans. Stability of field resistance depends on the buffering effect of the multigenic inheritance of this kind of resistance which counteracts the critical effect of the flexibility of the aggressiveness of P. infestans. Still the adaptation of the fungus to a dominating potato variety is possible. The late TOXOPEUS drew attention to the increase in disease attack in the old varieties Champion and Voran with increased acreage of the variety in question. Experience from the long term field testing in Mexico indicates no sudden changes of field resistance although in certain lines some decline has been observed.

Resistance due to hypersensitivity. At the beginning of this century Dr. SALAMAN at Cambridge and his colleagues could demonstrate that wild species were promising sources of resistance. S. demissum was employed in breeding experiments and proved to have a powerful series of resistance genes. Selections from this material remained free from blight for a number of years, but then they suddenly appeared susceptible. It was found that new races were easily produced by the fungus.

The results obtained from the first four R-genes and the sixteen different combinations of them showed that resistance of this form could only be of temporary value. Specialized races to match each gene combination were quick to appear. The R-genes thus failed to provide a permanent solution to the blight problem.

During the years of primary interest in breeding for hypersensitivity much knowledge has accumulated of the biochemistry and genetics of this type of resistance. European and Japanese workers have studied in detail the rapid death of an invaded cell or group of cells when the mechanism of hypersensitivity has been triggered by the infecting fungus. With the decreased value of the hypersensitivity reaction in breeding work this work will not be reviewed here.

Four genes for resistance were originally recognized in breeding material derived from S. demissum. Each gene is inherited independently in a monogenic-dominant manner. These genes were designated as R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub>. Including the recessive genotype 16 combinations are possible and differential host series of those 16 genotypes have been used for the identification of equivalent races of the pathogens. Five new R-genes from S.

demissum have been identified, which has expanded the possible combinations to 512. Several isolates previously designated as specific races on plants with the original four R-genes were found to have additional genes for pathogenicity. Now genes R<sub>10</sub> and R<sub>11</sub> have been recognized, which increases the number of combinations above 2000.

Genes similar to the R-genes obtained from S. demissum have been found in other Solanum species, e. g. S. stoloniferum, S. bulbocastanum, S. pinnatisectum, S. polyadenium.

## 2. Sources of resistance.

All present evidence point to the Mexican Solanum species as offering the best prospects in blight breeding. In Mexico and adjacent countries, both the parasite and its hosts have lived in close contact for a very long time with optimum prospects of the development of the complex of genes through natural selection necessary for a stable resistance. Although genes conferring resistance of the race specific type are very frequent they do not seem to contribute to the resistance necessary for survival of those species.

S. demissum is of outstanding value as a source of field resistance both concerning a low infection frequency and resistance once the fungus has entered the host. It is readily crossed with tuberosum and has been used more or less intensively by many breeders, originally as a source for R-genes but with a growing interest as a source of field resistance. Also the diploid Mexican species (S. phureja, S. verrucosum, S. pinnatisectum, S. cardiophyllum, S. trifidum and S. bulbocastanum) have attracted interest from potato breeders.

In several of these species there seems to be no clear distinction between a hypersensitive reaction to infection and one indicating a high degree of field resistance. Several reports indicate the possibility that, when no perceptible lesions are found, the fungus could have been completely excluded from the plant. The possibility of the existence of extreme resistance (not to be confused with race specific hypersensitive reaction) is not inconceivable in view of the fact that resistance to entrance (measured in infection frequency) is a factor with continuous variation from very high to very low, including none, number of infections.

Sibbing or selfing apparently improves the degree of resistance in the progeny over that of the parents of several of those species. Therefore a certain amount of "prebreeding" within the wild species might be desirable before hybridization with commercial tuberosum varieties. The potentials of breeding on a diploid level especially for the accumulation of polygenes affecting resistance has been advocated by PELOQUIN and HOUGAS, applicable to induced dihaploids of S. tuberosum but also to natural diploid species. Although those

wild species undoubtedly have a high value as sources of resistance, some of them may not be so easily available as breeding material because of difficulties in hybridization with S. tuberosum. The work of HERMSEN in Holland and several others to overcome this difficulty with S. bulbocastanum is promising.

While the Mexican potatoes are a major source of genes conferring resistance to blight, a certain fairly low level of field resistance is to be found in some species from the South American continent. Certain selections of S. tuberosum subsp. andigena and S. phureja of the Colombian potato collection have been reported to have a higher level of field resistance than clones of S. tuberosum. A certain degree of resistance has been reported by ROSS in S. commersonii, S. commersonii subsp. malmeanum, S. tarijense, S. acaule, S. spagazzinii, S. curtilobum, and S. tuberosum subsp. andigena, but not of a very high order. ROSS and ROWE have found resistance in three lines of S. microdontum subsp. gigantophyllum. Although some of those wild species carry R-genes as for instance S. vernei and S. kurtzianum the majority of them have resistance of a polygenic, non-specific type. Attention has also been drawn to andigena through the work at Pentlandfield. According to Dr. MALCOLMSON some of the parents derived from andigena now in use in a general breeding program is contributing towards field resistance. Laboratory and glasshouse studies on detached leaflets indicate some resistance to spread of P. infestans and its sporulation in leaves. The stems, however, appear to be extremely susceptible. Through the years Dr. MALCOLMSON has observed that infection with complex races of P. infestans gives a reaction similar to a major gene resistance, but without segregation suggesting major genes.

### 3. Breeding methods.

While breeding for hypersensitivity creates few problems in selection and evaluation work this has become a matter of major concern when breeding for field resistance. The ideal field resistant potato would be a variety with a low infection frequency combined with slow mycelial growth, rapid necrosis and low sporing capacity. The complex nature of field resistance and the predisposing effects of ontogenesis and environment must be taken into consideration in the selection work. It is also of importance to consider the requirements of the breeder for quick, reasonably accurate screening methods preferably in early stages of a breeding program, and with possibilities of handling large amounts of material at low costs. The work should be integrated with selection for other characters, e. g. yield, quality, resistance to other diseases. Observations which are tedious or influenced by plant variation and environment should preferably be postponed until later in the program when a lower number of breeding lines would be handled and more plants per clone available.

The screening test which is practiced in Svalov is based on the finding that plants with a low infection frequency also require a long inoculation access period (period of free water on the leaf surface and optimum temperature for spore germination and penetration) and that this character is manifested in the young seedling stage. The screening operates very similarly to the screening for hypersensitivity but is made on older plants and inoculum is prepared from races with a broad host range.

Selection for post infectional factors needs much more elaborate techniques and should preferably be made in subsequent clonal generations. Methods of screening clonal selections for field resistance with emphasis on the post-infectional factors have been practiced in Scotland, West Virginia and Colombia to mention a few places. Glasshouse grown plants are inoculated and incubated in humidity chambers and visual disease index measurements of the progress of the disease form the basis for the selection. Prerequisites for successful testing are:

- a) a large number of individual plants per clone,
- b) standardized growing conditions especially concerning soil fertility,
- c) use of races of P. infestans which would overcome R-gene resistance.

For detailed analyses of post infectional factors (fungal growth rate, rate of necrosis, generation time and sporing capacity) more or less complicated tests have been used either on detached or attached leaflets. Some workers have relied on single tests, others have made several different tests. The more characters included the more elaborate is the assessment and the fewer clones can be handled. On the other hand, accuracy may increase with more detailed studies. LAPWOOD in England analysed four criteria:

- a) fungal advancement estimated by measuring the radius of a lesion in mm up to the perimeter on the chlorotic area,
- b) extent of sporulation measured in mm of the sporing annulus,
- c) intensity of sporulation measured by visual rating,
- d) stem and petiole infection from inoculation of leaf axils.

All post infectional factors are considerably influenced by the level of mineral nutrition.

A rapid biochemical test method would be of great value for the assessment of the post infectional factors of field resistance. The peroxidase activity

test has offered some promise in this direction. A positive correlation has been found in S. tuberosum, valid also under the predisposing effects of light, leaf age, virus infection and certain mineral nutrition requirements. There is no definite proof that the peroxidase activity is directly related to or being the functioning mechanism of resistance.

Japanese workers (SAKAI and TOMIYAMA) fear that a high peroxidase activity in leaves may be an indication of late maturity. Since late maturity is closely correlated with resistance, they do not believe in a direct relationship between peroxidase activity and disease resistance. Although in general field resistant varieties are late in maturity there are differences in field resistance within the late maturity group. Besides, field resistant varieties have been found also in early maturing material. The correlation between field resistance and late maturity may have historical reasons as pointed out by van der PLANCK. Under natural field conditions early maturing varieties usually escape blight epidemics. Late maturing varieties have to face the full strength of the epidemic and therefore have been under considerable selection pressure.

Field selection can only be made under sufficient epiphytotic conditions. This is difficult to achieve when R-genes are introduced in the breeding material and the compatible races are not present. As mentioned, earlier breeders from many countries have taken advantage of the uniquely severe blight environment in Mexico.

The selection for an assessment of field resistance can then be carried out in the following way:

- a) in the seedling stage screening can be made of plants with a low infection frequency, that is plants demanding a long period of conditions optimal for infection. This does not include the post infectional factors.
- b) in subsequent clonal generations preferably when selection for other characters has reduced the number of clones, selection is made for rapid necrosis and low sporing capacity either by: screening in a glasshouse or in the field under sufficient epidemic conditions or by using biochemical methods.
- c) final checking of advanced breeding material and varieties is made by
  - 1) assessment under field conditions provided that compatible races occur naturally or can be introduced artificially or,



- 2) by assessment under laboratory conditions with determination of infection frequency by use of a quantitative inoculator and determination of postinfectious criteria such as lesion size and sporangium intensity.

One serious difficulty in selection and assessment of field resistance is the presence of R-genes. Since most of the field resistant selections of the moment are bred from S. demissum it is unavoidable that R-genes are involved in such material. R-genes also occur in S. vernei, S. kurtzianum and S. stoloniferum as mentioned earlier. There is no question about the value of those R-genes when they are functioning. They do however, cause difficulties in assessment of field resistance when no compatible races for the particular R-genes is available.

There are two ways of avoiding this difficulty. The first is not to use material with R-gene resistance. As mentioned before many of the South American species may have field resistance without R-genes involved. That would simplify screening and evaluation work in many locations. The second way is to assure the existence of compatible races of the fungus. This can be best achieved in Mexico which is a breeding ground for the fungus and the only place where the fungus is known to sexually reproduce.

MOOI has had experience of the complication of R-genes with the Dutch variety Multa, which originally was thought to possess a good degree of field resistance. However, two years after the release Multa was grown on a larger acreage than before and conditions for development of late blight were favorable. A new race of P. infestans appeared capable of attacking this variety. It was later described as race 1, 4, 10 and Multa had the gene  $R_{10}$  with a low level of field resistance.

MOOI also reports that much of the Dutch breeding material has in its parentage resistance factors  $R_3$  and  $R_{10}$ . However, races available in Holland or Scotland have not the combination of pathogenicity factors 3 and 10 and it is thus very difficult to assess the level of field resistance in this material.

The Dutch variety Prevalent is  $R_{10}$  genotype. It was sent to Toluca Valley in 1971 and ranked as highly resistant. However, in these areas of the Netherlands where race 1, 4, 10 was found Prevalent was also attacked and found to be less resistant than Alpha. This might indicate that also in Mexico not all compatible races are always available. MOOI suggests that all efforts should be made to promote a spread of compatible races in the testing field in Toluca.

#### 4. Adaptation to subtropical and other climatic conditions.

A number of observations suggest a variation in susceptibility to the disease with age of the potato plant. According to GRAINGER the potato plant shows two periods of susceptibility, one in the very young stage and another later, separated from the first by a period of vigorous foliage development characterized by resistance to late blight. The intermediate partial resistance

coincides with the time of the most rapid growth of the plant. At this time before the start of flowering the plants contract the disease only in the lowest leaves. At a later stage, corresponding approximately with flowering time, a change appears and the upper leaves gradually become susceptible. The increased resistance during the period of active growth of the foliage has been associated with slow growth of the parasitic hyphae after invasion but no change in rate of necrosis of the invaded tissues.

A correlation between field resistance and late maturity has been discussed by several authors. Many late maturing and field resistant varieties will have a prolonged period of vegetative growth with development of new axillary shoots. While the leaves of the main stems can be attacked quite early by blight and newly developed secondary shoots will be severely damaged, newly developed axillary shoots remain green with only a few sites of infection. A relationship between late maturity and field resistance seems obvious but does not exclude the combination early maturity and resistance. Many breeders claim to have such varieties. The combination late maturity and resistance is more pronounced in pure tuberosum material, than it is in hybrids with e. g. S. demissum.

A short day treatment of the potato accelerates its rate of development. Under very short days no or only a few axillary shoots are produced and no flowering occurs. Tuber formation starts even in usually late maturing varieties, and haulm maturity is hastened. A short day treatment also renders usually field resistant varieties susceptible to late blight.

In general photoperiod and ontogenetic predisposition seem to work in parallel. The hastening of development and ageing of the plant is not, however, the only reason for the effect of day length conditions of susceptibility to P. infestans. Inoculation experiments made by the author on potato clones grown under growth chamber conditions at photoperiods of fourteen hours and continuous light showed a marked effect on the susceptibility to blight. The difference in time of tuber set and maturity between the two photoperiods was very small, and both could be said to act as "long day conditions". Considerable differences however, were found in chemical composition. The main difference was a lower percentage of dry matter in leaves of plants grown under a fourteen hour day, while the gross composition of the dry matter was fairly unchanged. The data indicate that metabolic changes caused by the difference in photoperiod, but with little or no visible result in the rhythm of development of the plant, still cause considerable differences in susceptibility to P. infestans, susceptibility differences which cannot be explained by ontogenetic predisposition.

It is possible to stimulate the long day response by treatment of plants with gibberellic acid. Gibberellins may also influence the germ tube

length of spores of P. infestans. It has been observed by British workers when studying the infection of leaves by P. infestans that on resistant cultivars long germ tubes were produced before penetration in contrast to the immediate production of appressoria after germination on susceptible varieties. Exposure to short day conditions also gives a considerable decrease in natural gibberellins.

In northern Europe blight develops fairly late in the summer while in East Africa, e.g. Uganda, blight comes very early and kills a susceptible crop before it has bulked. BLACK finds little difficulty in breeding field resistant clones adapted to short day conditions and higher temperature in Kenya. Those clones which are too late for Europe seem to give best results. They probably have a degree of field resistance of such a dimension as to still be sufficiently resistant under short day conditions and behave with normal cropping features.

Blight tests under short day conditions rank the material more susceptible than it is under long day conditions. The field testing in Mexico is often found to be too severe for material intended for North European conditions. This can be avoided if the interpretation of the readings is based on experience from previous tests.

A sound base would be to have a set of standard varieties adapted to different climatic conditions tested under various blight conditions (including Mexico). Also needed is standard assessment key used by all breeders not only for assessing leaf area destroyed at a certain moment of attack but also for grouping varieties into different categories of field resistance. Although in general the accordance between blight readings in different countries (including Mexico) seems good, exceptions occur. WURSTER reported that some varieties known to be resistant were severely attacked in Uganda during growing seasons with heavy blight.

##### 5. Tuber resistance.

The need of tuber resistance to go along with foliage resistance has often been mentioned by breeders and pathologists. A variety highly susceptible in the leaves may be killed very quickly in a blight attack and a big mass of spores are produced within a short time, but the tubers may hardly be infested at all. A resistant variety on the other hand, keeps the fungus alive for a long time in a very inconspicuous way and the production of spores may go on for a long period. With no resistance of the tubers those may be damaged to a large extent. TOXOPEUS compiled data from several years of studies of more than 250 varieties on their tuber and leaf resistance. Although there was an obvious positive correlation, there was quite a large number of varieties which were highly susceptible in the leaves but moderately resistant in the tubers. Most observations on tuber susceptibility and

resistance are taken from field experiments, but reliable results depend on a good blight attack in the foliage, suitable weather conditions as well as soil conditions.

Screening methods for tuber resistance requiring few replicates have been suggested mainly along two lines. One group of tests measure the infection frequency after spraying of the undamaged tubers or pipetting inoculum on to the eyes. Another group of tests measures the rate of mycelial growth through the internal tissues of the tuber.

A test of the latter type has been described by LAPWOOD, who inoculated slices of medullary tuber tissue of a standard depth and as a criterion of resistance estimated the extent and density of aerial mycelium on the uninoculated surface after a number of days, or the time taken by the fungus to grow through the slice on to the other side. LANGTON has recently described the combination of the two groups. The criterion of resistance in his method was the frequency of infected cores of tuber tissue inoculated through wounded eyes combined with the average time taken by the fungus to appear on the uninoculated surface. LANGTON claims to have good correlation with field observations.

Experiments with unwounded tubers indicate that varieties very susceptible in the tubers under field conditions contract more infections especially through lenticels than do very resistant ones. Tubers of some tuber resistant varieties may develop many infections but the lesions will be arrested when still necrotic threads or after limited rotting of tissues. According to LAPWOOD the amount of rotted tissue separates susceptible from resistant varieties better than do numbers of tubers infected.

Tuber resistance has been found to increase with maturity. This has been attributed to changes in resistance of lenticels and not of eyes.

Changes in the content of phenolic acids and the activities of peroxidase and polyphenol oxidase have been associated both with the healing process after wounding and after infection with P. infestans. The content of phenolic acids increases after wounding and after infection. The increase is greater in resistant than in susceptible varieties. Peroxidase activity is increased, but the increase in polyphenol oxidase activity is still more pronounced especially in resistant varieties. SCHÖBER believes that P. infestans is stopped by a layer of suberine in the cell wall and the oxidation products of the phenolic acids in the cell. The ability of the tuber to synthesize suberine and oxidation products such as quinones after wounding and infection are probably of greatest importance in the mechanism of resistance to tuber blight.

However, other factors as indicated by LAPWOOD and associates, may also have influence on tuber susceptibility and resistance. The amount

of sporulation in the foliage differs greatly between varieties, with effects on the concentration of inoculum that reaches the tubers. The "run off" of rainwater from foliage depends on the growth habit of the variety; stem infections are common in some varieties and favours production of spores on a surface readily washed by rainwater. Tuber distribution in the ridge differs, some varieties from tubers on long stolons, others are clustered at the stem base.

RENEWICK has recently in an alarming report hypothetically discussed the presence of a teratogenic substance in blight infected potato tubers connected with two abnormalities in human beings: anencephaly and spina bifida cystica. BOYD has not been able to verify that a correlation exist between the frequency of blighted tubers and the malformations in babies examining Scottish data. Of major concern to us, however, is that RENEWICK also claims that there might be reason to suspect substances involved in the resistance mechanism to be teratogenic. From some selected papers he draws the conclusion that solanidine glycosides play a part in resistance to blight. Another group of antifungal compounds are the phytoalexins, which are synthesized by certain varieties in response to infection by certain strains of P. infestans. Those have been identified by Japanese workers as rishitin and phytuberin. There is at the moment no experimental evidence which, if any of those compounds, the solanidine or phytoalexins include the actual teratogene.

#### B. The fungus

The mating types have been described in P. infestans designated as A<sup>1</sup> and A<sup>2</sup>. A<sup>2</sup> seems to be restricted to Mexico while A<sup>1</sup> has been found in North America, Western Europe, the West Indies, (and according to a personal communications by Drs. J. Galindo and H. Niederhauser also in Brasil, Colombia, Peru, Japan and parts of Africa). Both mating types thus exist in Mexico and are usually present in a ratio near 1:1. They are compatibility types, and not morphological sex types. Each isolate is bisexual but self-incompatible. The degree of maleness and femaleness varies; some isolates of each compatibility type act as strong males and some as strong females, some being intermediate in relative sexual strength.

When gametangial hyphae from the two compatibility types approach each other on lima bean agar usually hyphal attraction is seen. When they meet, the oogonial hypha penetrates the tip of the antheridial hypha. Gametangia formation can also be induced by the Trichoderma method. Only one compatibility type is needed and the offspring is the result of selfing.

In a recent paper SANSOME and BRASIER report that in crossed as well as selfed material two nuclear divisions normally occur in the gametangia, and thus normal meiosis occur prior to gamete formation. The chromosome number from counts of first metaphase configurations is 16 - 20 (2 x (9± 1)).

According to these data P. infestans is diploid in its vegetative state.

There has been, however, some controversy whether the asexual stages are haploid or diploid. Most of the species of the Oomycetes that SANSOME has studied have been homothallic species of Phytophthora. There has been some speculation whether the homothallic species are diploid and the heterothallic species are haploid. The present results of SANSOME and BRASIER are supported by the cytological findings of GALLINDO and ZENTMYER in their work with the species P. drechsleri which also appeared to be diploid in its asexual stages. Results from inheritance studies, however, indicate more strongly a haploid state.

With increased interest on developing varieties with a high degree of field resistance the thrust is to have a more stable type of resistance. Although no drastic changes in pathogenicity on field resistant varieties have occurred for several decades the report of GALLEGLY showing presence of aggressive isolates of P. infestans highly pathogenic on field resistant tomato varieties is alarming.

Several investigations give evidence of intraracial variation in aggressiveness among isolates of P. infestans. It is well established that isolates lose aggressiveness after prolonged culture on artificial media. Aggressiveness can be restored after culturing of the isolate on leaves or tubers of the variety from which it has originally been obtained.

Evidence also points to the fact that isolates with several R-gene specific genes are less aggressive than isolates with few or none of these genes. Passage through a susceptible host usually results in the disappearance of the complex races. The build up from an isolate with one pathogenicity factor to several, e. g. race 1 to race 1, 4 has also been described.

The mode of inheritance of pathogenicity is at the moment very controversial. Mutation is generally accepted as the mechanism giving rise to new race characters.  $R_4$  genotypes frequently appear spontaneously in almost any pathogenic isolate of P. infestans. Very few have succeeded, however, to induce mutations artificially. If P. infestans is diploid in its vegetative state, as the cytological studies of SANSOME indicate, recessive mutations will not be expressed in the original colony, which may explain the failure to induce mutations artificially.

DENWARD, and independent of him, BENNET are of the opinion that extranuclear entities, structures that can be defined as episomes, may replicate and distribute in the hyphae. CATEN and JINKS suggest that variation in rate of growth and sporangium production is under cytoplasmic control.

C. Expected contributions of CIP.

In the late blight program of CIP priority should be given to projects intended to create a basis for rational blight resistance breeding. One of the reasons for the planning meeting, is to discuss and put forward questions, which have not been solved and need attention. Those questions may fall in various fields, e. g.:

1. The saving of genetic material of potential value for future late blight resistance breeding at the moment in danger of genetic erosion.
2. A better understanding of late blight resistance, its mechanisms, inheritance and factors influencing its expression.
3. The continuation of the international late blight test in Mexico and the most efficient way to make use of the material and knowledge available there for the future.
4. The extension of knowledge, experience and material to national breeding programs interested in blight resistance.

**4**

# ***Bacterial Wilt***



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## CONTENTS

	Page
I. SUMMARY OF RECOMMENDATIONS	187
II. INTRODUCTION	188
III. SUMMARY OF CURRENT RESEARCH	189
IV. DEFINITION OF PROBLEMS	195
V. DISCUSSION OF OBJECTIVES	196
VI. RECOMMENDATIONS	198

I. Summary of Recommendations  
for Developing Bacterial Wilt Resistant  
Potato Clones, with Tentative Dates of Initiation

CROSSES (Jul. - Aug. 73)

↓  
Mass screening under controlled environment at Wisconsin  
(Sept. - Dec. 73)

↓  
Survivors grown to maturity  
Clones yielding less than 8 tubers discarded  
distribution of tubers (Feb. '74)

6 to CIP, Peru

2 kept in Wisconsin

Excess, 4 tuber  
field tests in  
Colombia, Costa  
Rica. (May-Nov. '74).

2 for late blight  
test by inoculation  
in screenhouse.

4 for wilt test  
in field.

Tubers from resist-  
ants to:

(Results)

→ Susceptibles eliminated

Increase for back-up  
collection

↓  
Wilt test in Brazil,  
Colombia, Costa Rica, etc. \*

↓  
Excess tested to international  
isolate collection at CIP.

↓  
Distribution to other  
countries (e.g. Nigeria)

2 tubers to Toluca  
Mexico, for blight test.

\* Countries that can receive tubers from Peru.

CENTRO INTERNACIONAL DE LA PAPA (CIP)  
POTATO BACTERIAL WILT PROJECT PLANNING CONFERENCE

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II. INTRODUCTION

A program to evaluate *Tuberosum* clones for resistance to bacterial wilt (*Pseudomonas solanacearum*) was conducted at North Carolina State University by Haynes & Nielsen from 1947 to 1960. Nearly 9000 clones were tested but only moderate levels of tolerance were found. Resistance was discovered by Thurston and Lozano among the Phureja in the Colección Central Colombiana. With these stocks a program was initiated in 1967 by Rowe and Sequeira at Wisconsin to develop germ-plasm that would be useful to potato breeding projects in countries where bacterial wilt was a problem, and to investigate the inheritance of resistance. A first series of crosses were made in 1968 with the objective of combining wilt resistance and acceptable horticultural type. A second series of crosses in 1970 combined these characteristics with late blight (*Phytophthora infestans*) resistance derived from cultivars developed at Toluca, Mexico. Progenies sent for field testing to numerous countries performed well in many instances, but reliable results were not forthcoming from several of these. With the birth of CIP and its assumption of responsibility for the coordination of this project (with linkage to Wisconsin) this Potato Bacterial Wilt Project Planning Conference organized by CIP in conjunction with the University of Costa Rica, provides an opportunity to expedite the evaluation and utilization of Phureja wilt resistance around the World.

### III. SUMMARY OF CURRENT RESEARCH

#### A. COLOMBIA

In 1968, ICA received from Wisconsin the first set of 1086 clones to be tested against P. solanacearum. This material was planted in Medellín, Antioquia Department (1500 m. elevation) and Popayán, Cauca Department (1800 m.). Most of these clones were highly susceptible to P. infestans, but 234 were selected as resistant to P. solanacearum and P. infestans. These were replanted in Popayán in 1969-1970 in a plot where the check, ICA Puracé, had 74.1% wilted plants in 1969. Selection was based on P. solanacearum resistance and tuber type; 38 clones were selected, and replanted in 1970. At the end, 11 clones had less than 20% infection and 6 clones had less than 10%; they have been increased at Tibaitatá and San Jorge. The best clones (P-7 and P-13) could become new varieties.

In 1970, ICA received a set of 672 "BR" clones which were planted in Popayán in 1970, 1971 and 1972, with selections each year. This material will be harvested in late December 1972. In 1970, we also received material from the Potato Breeding Program of ICA. This material was the progeny of crosses between local commercial varieties and S. phureja (C. C. C. 1449). After four replantings, there are some with high P. solanacearum resistance and good tuber characteristics.

#### B. COSTA RICA

1. Field tests. In Costa Rica, potatoes are affected by bacterial wilt at elevations below 2000 m. Two sets of hybrid material were evaluated in Costa Rica; 1916 clones were received in 1968 and 1350 "BR" clones in 1969. They were planted in a sequence of different sites, infested and non-infested, and selected on the basis of tuber type and resistance to Bacterial Wilt. In 2 years (5 plantings) clones were reduced from 3270 to 22. In the fourth planting the check had 90% wilt while selected clones averaged 15%. In the last they had 22% and 4% wilt, respectively. In 1971 some of the surviving clones were grown from healthy tubers in a growth chamber at the University of Wisconsin and stem - inoculated with two virulent isolates of P. solanacearum. Most were resistant to S-206 and a few to S-213 (the most virulent in the Wisconsin collection). These tests confirm that field selection, although laborious and time-consuming, can screen out resistant material.

2. Seedling screening for large progenies. In 1970-71, a procedure was developed at Wisconsin to screen out large progenies at the seedling stage. In its final form, it consisted of: a) planting true seed 3.2 cm apart in flats of Jiffy - mix; b) growing at 22°C for 20 days; c) allowing the mix to dry out; d) pouring 5 liters of inoculum consisting of isolates S-207 and S-123 at a concentration of 2 and 1 x 10<sup>5</sup> cells/ml, respectively, in water; e) cutting the roots immediately with a knife run between and across the rows of seedlings; f) incubating at 28°C for 2 weeks; and g) transplanting survivors to a greenhouse. Most susceptible seedlings were eliminated in the first few days, and some more came down in the greenhouse. By this method, 7851 seedlings were reduced to 315 in 5 months. Stem-inoculation tests with isolate S-206 on a random sample of surviving clones (labelled MS for mass screened) indicated that about 80% of them were in fact resistant (L. Sequeira). A large sample of them has been increased in Costa Rica and will be field - tested in 1973.

#### C. HONDURAS

In Honduras, potatoes are grown at elevations ranging from 1500 to 1900 m. where wilt limits production.

The following clones were selected on the basis of high yield and resistance to bacterial wilt: T-1, U-7, 8-5, 4-2, N-35, U-6, M-4, T-12 and P-11. The last two were resistant in a second test. These results are considered tentative. Work was interrupted because of inadequate means to continue.

Clones U-7, 8-5 and N-35 have been supplied again for testing in 1973. In addition BR clones 62-3, 62-5, 63-65, 63-76 and 73-4 will be tested. A range of 10 to 60 tubers of these clones have been received.

#### D. NIGERIA

The following material has been tested at infested sites of 800-900 and 1400 m. elevations: a) 17 S. phureja x S. tuberosum hybrid clones; b) 230 "BR" single hill tubers.

Results: a) A-1 and 311.5 remain resistant to bacterial wilt. b) The selection BR 63-5, (A-1 x Atzimba) has good resistance to Bacterial Wilt and Late Blight; it is a good yielder and acceptable agronomically; 700 lbs. are presently being multiplied for possible distribution to growers.

The selection BR 63-18 also has good Bacterial Wilt resistance but would be unacceptable to growers.

Objectives: To develop, as quickly as possible, a reasonable clone that has an essential amount of resistance to Late Blight and Bacterial Wilt, with more emphasis on the degree of field resistance than tuber characteristics: shape, size, color, etc.

#### E. PERU

The work reported on has been executed by Ing. Isaias A. Herrera, who began this work as a graduate student and is now a researcher with the Ministry of Agriculture. Selection was done at Huambos, Cajamarca at 2350 m. elevation.

Thirteen S. phureja clones received from Wisconsin were tested, with five being resistant.

Also, 49 S. phureja x S. tuberosum clones were tested in 5 "1-tuber" randomized plots, and 355 tubers of 12 families (BR 60 to 73) were tested.

Tubers from the above tests were harvested from apparently resistant clones and replanted in 5 "1-tuber" plots during three consecutive growing seasons. Clones were eliminated if they showed symptoms of wilt, or tuber rot at harvest or after storage for 6 weeks.

The first group of 49 clones yielded 4 resistant. The 355 BR tubers dwindled down to 20 clones. These represent a selection of about one resistant among 16 clones. Six of these 24 clones have been selected as potential varieties and are being multiplied for large scale tests.

#### F. WISCONSIN

1. A new approach to resolve the specific resistance needs of a country is being tried. The seedling inoculation technique is being used to select clones with resistance to specific local strains. Nearly all the clones that were originally sent to Ceylon were reported to be susceptible; progenies are now being tested against Ceylon strains in the hope of selecting clones of potential value to that country (Mark Hastreiter).



2. Utilizing the seedling inoculation technique to study the genetic basis of disease resistance, Luis González showed that the method has potential, but is subject to problems - i.e. the concentration of inoculum, the age of the seedlings, etc. are very critical. At present, we are attempting to determine how the different genes for resistance (RAB, RDE, etc.) are interrelated to provide resistance to wide groups of strains. Our approach is to determine resistance: susceptibility ratios in hybrid progenies of R x S, R x R and S x S crosses involving parents whose genotypes are known, based on published information. It is obvious that there is not a specific gene combination for each specific isolate of the bacterium, but there must be groups of isolates which respond similarly to gene products from each group of resistant genotypes (J. C. Zalewski).
3. Effect of light on resistance. There is consistent evidence that a low light regime does reduce resistance (Frank Vojtik).
4. Chemical nature of disease resistance. In preliminary studies, a growth inhibitor of *P. solanacearum* was detected in crude potato tuber extracts. Work was initiated by Jim Zalewski and L. Sequeira determine the possible involvement of this inhibitor in resistance to bacterial wilt.
  - a) The compound is preformed - inoculated with *P. solanacearum* did not increase levels of the inhibitor present in tuber, stem, and leaf tissues of both *Solanum phureja* and *S. tuberosum*. Inhibitory activity of crude extracts from 3 *S. phureja* clones was consistently higher (2-3 times) than that of extracts from *S. tuberosum* Russet Burbank. Extracts from clones held at 1800 ft-c for 1 week were more inhibitory than similar extracts of plants grown at 600 ft-c.
  - b) The crude extracts had the characteristics of an antibiotic. Non-lethal levels of the inhibitor increased the lag period and decreased the growth rate. High concentrations were bactericidal; low concentrations were bacteriostatic.
  - c) Concentrations of the inhibitor which inhibited *P. solanacearum* (K-60) were stimulatory to *Erwinia carotovora* and *E. atroseptica*. This differential inhibitory effect may explain why *P. solanacearum* is incapable of rotting potato tubers as rapidly as the soft rotting Bacteria.
  - d) Partial purification by paper and column chromatography yielded a compound with UV absorption maxima at 264 nm in

its biologically active form and 282 nm after acid treatment. A quantitative assay for this compound, however, failed to yield any correlation between its content in extracts from various S. phureja and S. tuberosum clones and resistance to P. solanacearum. It appears that the 264-282 nm compound is not the major inhibitor in crude extracts.

Work on the chemistry of the 282 acid product (mass spec) indicates a molecular weight of 206 and the formula  $C_{11}H_{10}O_4$ .

5. Genetics and breeding. Two of the objectives of the Wisconsin program on bacterial wilt have been to study the inheritance of resistance to develop germ plasm that could be used in breeding programs. Each of these objectives has been met to some extent and the results should be considered when making plans for future work.

Studies based on tests conducted in growth chambers have led to the development of a hypothesis that dominant genes at independent loci confer resistance. Only two isolates (K-60 and S-123) of P. solanacearum have been used in extensive tests. The inheritance of resistance of these two isolates is believed to be controlled by two dominant and independent genes for each isolate and by a common gene. While this hypothesis needs further testing, it does provide some insight on the proper procedures to be used in a breeding program.

The development of germ plasm has involved the hybridization of the resistant Phureja clones with various diploid and tetraploid clones of Tuberosum. Stocks in the form of tubers and seed have been sent to many countries where they have been exposed to a range of test conditions. In view of the complications of field tests, it looks like the number of resistant clones that have been found is impressive. It is further proof that a high level of resistance can be transferred from Phureja. It is also indirect evidence that resistance is rather simply inherited.

The genetic and field test data that are now available indicate that each location will need to test clones to determine those that are resistant under the conditions at that location. Only rarely will a clone be resistant at several locations. However, a sample of clones that are resistant at one location should have a higher frequency of resistant clones at another location than an unselected

sample. Crossing local cultivars with clones with resistance to bacterial wilt will be the fastest way to produce adapted, resistant clones.

It should also be possible to combine resistance to bacterial wilt and resistance to other diseases but this should be done on a priority basis. Sources of resistance might include the wild species chc and sto or advance selections that have the resistance factors that are needed.

We are fortunate that resistance to bacterial wilt has been found in a cultivated Solanum species that is highly fertile and easily crossable with Tuberosum. The lack of any crossability barriers and the simple patterns of inheritance that have been found should simplify efforts to combine various parental stocks. Adequate testing and proper maintenance of selected clones are the next problems to be solved.

6. Results of field tests. The results of field tests can be provided in two ways; either by country or by clone. A brief summary of the results from countries that are not represented in the workshop is given.

Brazil - Drummond found resistance in 8-8, 8-26, D-62, D-27, D-70, DA-1, DC-1, E-82, G-81, G-92, GC-3, J-86, P-3, P-15, T-2, and V-7. He also found resistance in early BR families (2, 4, 5, 6, 7, 20, 21, 22, 23). Raúl Ribeyro found resistant plants in progenies that involved N-68 and A-1 as sources of resistance. He also found that the clones BR 15-4, BR 18-16, BR 28-16, and BR 54-1 were resistant, especially BR 18-16. The most promising individuals that he has found came from the cross of A-1 x Greta.

Ceylon - One report of resistance in Phureja crosses. No more work being done.

Fiji - First results all negative. No data yet on next test.

India - No results have been reported to CIP. Many stocks have been sent.

Indonesia - No data received yet. Tests underway.

Kenya - Very brief reports but these have indicated that some Phureja plants have resistance in their tests. 1386.15 x 1339.8 was a good source.

Mauritius - No data received yet except that 8-18 is susceptible to late blight. Tests underway.

Philippines - In a test of seedlings, A-1 x Anita gave the highest percentage of survivors. Greta x P.1 also did well. No data on recent tests.

Requests have been received recently for stocks for Guadalupe, Madagascar, and Guatemala.

Mexico - There is no Bacterial Wilt problem in Mexico. It is not clear whether this is due to failure to be introduced or to adverse climate. They would rather keep it out than find out. Late blight testing is hindered by a stringent quarantine law. A request for exceptions to be made for research purposes is under study by Mexican authorities. Further testing must await new regulations.

#### IV. DEFINITION OF PROBLEMS

It was proposed that future policy must provide an answer to the following questions:

- a) How many clones to maintain in Wisconsin, so as to avoid overloading their storage, and growing facilities?
- b) Can interested countries do their own screening from true seed, thus liberating the bacterial wilt project from the maintenance of all but the most promising parental material?
- c) How to tailor future crosses to the needs of each country?
- d) Is there a possibility or need to incorporate resistance to diseases other than Bacterial Wilt and Late Blight?
- e) How to incorporate the seedling screening test into the general scheme of production and testing of new materials?

- f) How to be certain that P. solanacearum will not be exported in tubers from seedling - screened clones?
- g) Is it possible to run a Late Blight seedling test first and then move on to field-testing for Bacterial Wilt?
- h) How to move clonal material out of countries like Mexico and Peru to countries with increasing quarantine regulation?
- i) Can field testing for Bacterial Wilt be organized so as to obtain results in less than 2 years?
- j) How to assure a reliable feed-back of information from countries receiving materials for testing?
- k) Where (other than Wisconsin) can clones in the process of being screened be maintained and increased free of virus, to be distributed to requesting countries once their resistance is known?
- l) What alternative is there to late blight screening in Mexico where new quarantine regulations may impede or delay future work?

## V. DISCUSSION OF OBJECTIVES

### A. Priorities of an expanded program.

- 1. The objective of the program has been to provide useful wilt-resistant clones to countries. It was not, necessarily, to supply fully improved and adapted varieties.
- 2. To find out whether the countries' diverse requirements can be satisfied by just the breeding program carried out in Wisconsin, each country must test extensively a number of advanced clones. This will be the basis for deciding which ones should become varieties or serve as parents for further breeding.
- 3. In cases where one of the desirable parents for further crosses is strictly a local variety, the breeding will have to be done locally (Colombia, Peru and probably Brazil could do it; Costa Rica, Honduras and Nigeria are not likely to do it).

4. The general guideline for future breeding work recognizes that continuous commercial planting in infested soil is undesirable anywhere; rather, the importance of clean seed and crop rotation as complements to wilt-resistance must be recognized. Moderate levels of resistance are probably adequate for cool potato growing regions.
5. No search for better sources of resistance than the present clones of S. phureja is contemplated.
6. There is a need to improve the seedling mass-screening procedure to cope with local needs, as in the case of the particular virulence of the bacterium in Ceylon and Fiji. This "tailor-made" screening can be applied to the seed from specific crosses.
7. Simultaneous field testing and multiplication do not work. Maintenance and increase should be in wilt-free areas. There is a need for either a seed-multiplication program in each country or regional centers that can supply neighboring countries with commercial amounts of seed.

B. Anticipated role of CIP.

1. International coordination to improve the flow of information and materials.
2. Establishment of regional coordinators through which efforts on Bacterial Wilt could be channeled.
3. Supplemental funding for cooperative projects in instances when a scientist is better able to conduct a specific item of research than can be performed by CIP in Perú, but he has funding limitations.
4. Possibility for multiplication of valuable stocks in double-screened houses in Perú.
5. Sponsorship of technical meetings, such as a short course in plant breeding to induce Bacterial Wilt breeding work by interested scientists trained in other disciplines or crops.
6. Investigations on specific aspects, such as the importance of latent lenticel infections as a masked means of spread of P. solanacearum from seed lots.

## VI. RECOMMENDATIONS

### A. Development and distribution of materials for testing.

1. Maintenance of clonal material at Wisconsin will be concomitant with proof of their potential usefulness. A list was drawn of the material that will be kept in the back-up clonal collection.
2. The seedling screening test will continue to be done in Madison but it is recommended that it be tried under different set-ups in Perú, Colombia, Costa Rica, etc.
3. Hopefully, new material surviving the seedling test will not be increased again in Wisconsin until Blight susceptibility information has been received and thinning carried out.
4. Sets of 6 tubers of each new clone will be shipped to only one country (Perú) for parallel testing against Blight and Wilt (as opposed to sending sets of 2 tubers to each of several locations). To diminish the risk of eliminating in Perú materials with possible value in other countries, clones will be discarded only when a 4-tuber Wilt test indicates high susceptibility in many other places). Also, surplus tubers of high-yielding clones will be sent to a second or third country.
5. Collaborating countries will receive clonal material from Perú when allowable. Otherwise, they will be supplied from Wisconsin. A second Late Blight test, at Toluca, will be carried out if quarantine regulations permit.

### B. Field Tests.

1. Maintenance of test areas with high inoculum level is emphasized. However, it may be advisable to rotate with cereals to prevent the build-up of other diseases and to eliminate volunteer potatoes.
2. As nearly uniform planting dates as possible in countries receiving material for testing is desirable. April-May is the time consistently given as best or next-best by participating countries.
3. Minimum guidelines for field tests include:
  - a) Appropriate randomization of the clones being tested. This is best done before the planting date.

- b) Use of susceptible and resistant checks, preferably the seed tubers should be produced on the same land as the test clones.
- c) Designs that can be statistically analyzed. Replication is necessary, plots should be randomized.
- d) Moderate spraying for Late Blight control.

C. Maintenance of stocks-quantity and location.

- 1. Each country will have to maintain as much selected material as its facilities (storage and disease-free increase) will allow. Wisconsin will only maintain a relatively reduced number of clones of general usefulness.
- 2. National seed-production programs are a definite requirement for maintaining prospective resistant varieties and increasing them to commercial levels.

D. New crosses and diversity.

- 1. For the moment no attempt to incorporate resistance to disease other than Bacterial Wilt and Late Blight is contemplated. It is expected that adequate seed programs will take care of virus problems.
- 2. In case specific crosses were needed, the arrangements can be made directly with P. R. Rowe in Wisconsin, depending on the availability of parents.
- 3. The need for better sources of resistance to Bacterial Wilt will depend on the success of the available levels and the relevance of potato cultivation in tropical areas with high disease potential.

E. Release of cultivars.

- 1. When a variety is released, there will be a publication about it, including appropriate recognition to contributing institutions, in addition to the official recording required by the institution concerned. Records will also be kept by CIP and Wisconsin. CIP will coordinate the naming of a new variety when the same clone is selected in more than one country.



2. The person making the selection (not the one who made the cross) is recognized as "the breeder" of the cultivar and is responsible for it, including the maintenance of the foundation stock.

F. Coordinated program to be implemented in 1973.

1. On the basis of the above mentioned guidelines, a procedure for the development of new materials was agreed upon, and is shown schematically on the first page. This plan will be implemented in 1973.
2. Uniform testing of selected advanced stocks.
  - a) A list was drawn of 10 outstanding 1968 and "BR" hybrid clones, which appear to be resistant to Bacterial Wilt and, to some extent, have adequate tuber type.
  - b) These 10 clones will be increased in Wisconsin, first in the greenhouse, then in the field. Thirty tubers per clone, along with 30 tubers of Kennebec (US check) will be sent about October 1973 to participating countries, to confirm their worth as resistant parents or potential varieties.
  - c) Each country will add a local check (adapted but susceptible) when feasible. Design will be according to each collaborator, but the randomized block is recommended. There will be 5 replicates of 6 tubers each per entry. Data should be secured and 35 tubers saved for retesting in each of three successive seasons along with subsequent shipments of tubers from Wisconsin (unexposed to the bacterium). The data recorded on forms prepared by R. Rowe, should be sent to him.
  - d) The following data should be recorded on special forms:
    - i) Emergence, one month after planting (examine to determine if non-emerging plants were infected by P. solanacearum or other pathogen or pest).
    - ii) A minimum of 2 readings of wilting plants during the growing season, giving plus (+) or minus (-) readings to each plant (one wilting stem means a (+) reading, unless wilt is very slight). Plants recorded (+) should be pulled out and left on the hill site.

- iii) Late Blight readings on the standard 1-5 scale.
- iv) Tuber scoring for Brown Rot, also on a per-plant basis: at harvest, a (+) reading is given to any plant with even one single confirmed infected tuber; then each (-) plant is stored for one month in an individual bag at room temperature and scored again, (+) or (-).
- v) Yield (weight and number of tubers per plot) and tuber type (good, medium or poor).

**5**

# ***Nematode Control***

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The following members of CIP staff also attended the Planning Conference:

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Head, Dept. of Breeding and Genetics

Dr. E. French

Head, Dept. of Pathology

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Director of Research

## CONTENTS

	Page
I. AGENDA	206
II. INTRODUCTION	213
III. PRIORITIES FOR NEMATODOLOGY RESEARCH	214
IV. RECOMMENDED POTATO CYST NEMATODE RESEARCH	217
V. RECOMMENDED ROOT-KNOT NEMATODE RESEARCH	227
VI. RECOMMENDED FALSE ROOT-KNOT NEMATODE RESEARCH	230
VII. RECOMMENDED ROOT LESION NEMATODE RESEARCH	233
VIII. RECOMMENDED RESEARCH ON NEMATODE INTERACTIONS	236
IX. RECOMMENDED FACILITIES FOR RESISTANCE TESTING	239
APPENDIX I	241

CENTRO INTERNACIONAL DE LA PAPA  
PLANNING CONFERENCE ON NEMATOLOGY RESEARCH

AGENDA

Monday, February 11

- |       |   |
|-------|---|
| 9:30  | Introduction of Participants;   |
|       | Overview of Objectives of the Planning Conference -<br>Dr. R. L. Sawyer, Director General                                     |
| 10:15 | Coffee  |
|       | I POTATO CYST NEMATODES   |
| 10:30 | Brief Comments on Part I of Position Paper:<br>"Potato Cyst Nematodes" - Dr. W. F. Mai  |
| 11:00 | Status of knowledge concerning pathotypes of<br><u>Heterodera rostochiensis</u> and <u>H. pallida</u> -<br>Dr. F. G. W. Jones |
| 11:15 | General discussion - Pathotypes of potato cyst<br>nematodes - identification, classification and<br>economic significance.    |
| 12:15 | Lunch at La Molina  |

Monday afternoon

- |      |   |
|------|---|
| 1:30 | Classifying and determining the distribution of<br>pathotypes of <u>H. rostochiensis</u> and <u>H. pallida</u> in the<br>Andes - Dr. María de Scurrah |
| 1:45 | General discussion - Development of a CIP program<br>to classify and to determine the distribution of<br>Andean pathotypes of potato cyst nematodes.  |

- 2:30 General discussion - Evaluation of crop loss by nematodes - experimental designs and statistical evaluation.
- 3:00 Coffee
- 3:15 Status of known sources of resistance and tolerance to the potato cyst nematode - Dr. H. Ross
- 3:30- 4:15 General discussions - Methods of evaluating resistance and tolerance; attributes of resistant and tolerant potato clones and cultivars.

Tuesday, February 12

- 8:45 Searching for new sources of resistance and tolerance to the potato nematode - Dr. R. Schaefer
- 9:00 General discussion - Development of a CIP program to screen for resistance and tolerance to the potato cyst nematode.
- 9:45 Maintaining a pathotype and test plant collection at CIP - Dr. K. Evans.
- 10:15 Coffee
- 10:30 Summary discussion - Potato cyst nematodes
1. CIP Quarantine regulations
  2. Status of taxonomy
  3. Collecting and sampling techniques
  4. Interaction with other disease organisms
  5. Other aspects
- 12:15 Lunch at La Molina



Tuesday afternoon

II. ROOT-KNOT NEMATODES, Meloidogyne spp.

1:30

Brief Comments on Part II of Position Paper:  
"Root-knot Nematodes" - Dr. B.B. Brodie

1:45

The root-knot nematodes as pathogens of potatoes  
Dr. B.B. Brodie

2:00

Summary discussion - Meloidogyne spp.

1. Tropical and sub-tropical distribution
2. Collecting and sampling techniques
3. States of taxonomy
4. Interaction with other disease organisms
5. Other aspects

3:00

Coffee

III. ROOT-LESION NEMATODES, Pratylenchus spp.

3:15

Brief Comments on Part III of Position Paper:  
"Root-lesion Nematodes" - Dr. W.F. Mai

3:30

The root-lesion nematodes as pathogens of potatoes

3:45 - 4:30

Summary discussions - Pratylenchus spp.

1. Tropical and sub-tropical distribution
2. Collecting and sampling techniques
3. Status of taxonomy
4. Interaction with other disease organisms
5. Other aspects

Wednesday, February 13

IV. FALSE ROOT-KNOT NEMATODES, Nacobbus spp.

8:45 Brief Comments on Part IV of Position Paper -  
Dr. - B. B. Brodie.

9:00 The false root-knot nematode (Nacobbus spp.)  
as a pathogen of potatoes - Dr. P. Jatala

9:15 Summary discussion - Nacobbus spp.

1. Tropical and sub-tropical distribution
2. Collecting and sampling techniques
3. Status of taxonomy
4. Interaction with other disease organisms
5. Other aspects

10:15 Coffee

10:30 General Discussion - Nematode Ecology

1. Influence of soil type
2. Influence of soil temperature and moisture
3. Influence of elevation on distribution
4. Host range
  - a) Heterodera spp.
  - b) Meloidogyne spp.
  - c) Patrylenchus spp.
  - d) Nacobbus spp.
  - e) Trichodorus spp.
5. Parasites and predators

12:15 Lunch at La Molina

Wednesday afternoon

1:30 General Discussion - Breeding for nematode resistance and tolerance in potatoes - a CIP program.

1. Success in other crops
2. Relative tolerance of various potato clones
3. Methods of screening and evaluation

2:45 Coffee

3:00 General Discussion - Nematode control in potato crops in developing countries.

1. Cultural practices
2. Chemical control

3:45 A suggested nematology research program for CIP - Dr. C.A. Huijsman.

4:00- 4:30 General Discussion - Format of preliminary draft report leading to specific recommendations - Dr. O. T. Page.

Thursday, February 14.

Preparation of Preliminary Report by Drs. Brodie, Mai and Page. This Report will be presented to all participants of the Planning Conference on Friday, February 15, for discussion and development of specific recommendations to guide the nematology program at CIP.

Friday, February 15

8:45 Cooperation between CIP and the National Potato Research Centers; role of CIP in the development of an international system of pathotype recommendations - Dr. E. French.

9:00

I. Potato Cyst Nematodes:

Discussion and development of recommendations re:

1. Surveys in Peru and designated developing countries; identification and taxonomy.
2. Control : a) Breeding for resistance and tolerance  
b) Cultural practices  
c) Chemical
3. Duration of study period for each recommended project.

10:15

Coffee

10:30

Potato Cyst Nematodes - continued

12:15

Lunch at La Molina

Friday afternoon

1:30

II. Potato root-knot nematodes

Discussion and development of recommendations re:

1. Surveys in Peru and developing countries
2. Control - breeding, cultural, chemical
3. Duration of study period for each recommendation

2:15

III. Potato root-lesion nematodes

Discussion and development of recommendations re:

1. Surveys in Peru and developing countries
2. Control - breeding, cultural, chemical

3. Duration of study period for each recommendation

3:00 Coffee

3:15 IV. False root-knot nematodes

Discussion and development of recommendations re:

1. Surveys in Peru and developing countries
2. Control - breeding, cultural, chemical
3. Duration of study periods of each recommendation

3:45 Concluding remarks by Dr. R. L. Sawyer

## II. INTRODUCTION

Nematodes pathogenic to potatoes cause serious crop losses but much of this damage is unrecognized. Because nematodes attack roots (sometimes tubers) and there are no diagnostic symptoms on above-ground parts of the plant, nematode damage is often attributed to other causes. High population densities of nematodes in the soil cause unthrifty top growth which resembles symptoms resulting from poor root growth which can be due to many factors. Lower densities in the soil cause no overt above-ground symptoms but may reduce tuber yields. As the world population increases, soil suitable for potato culture will become more scarce. Consequently, potatoes will be grown more frequently on the best potato land and nematode damage to potatoes will increase dramatically.

The potato cyst nematode (Heterodera rostochiensis and H. pallida), root-knot nematodes (Meloidogyne spp.), root lesion nematodes (Pratylenchus spp.), and false root-knot nematodes (Nacobbus spp.) are considered to be the most important nematodes which attack potatoes. Other nematodes, including Trichodorus spp. which transmit tobacco rattle virus to potato, are known to be parasites of potato.

The potato cyst nematode is the most feared as it causes one of the most damaging diseases of potato. Although populations of this nematode do not increase as rapidly as fungal and bacterial pathogens of potatoes, once the nematode is well established in a potato-growing area it is impossible to eradicate. It is costly and difficult to keep soil populations below damaging levels and to prevent spread. This nematode occurs in most potato-producing areas of the world and probably will be discovered soon in the rest of them. The mature, swollen females (cysts) in which eggs may remain viable for 15 or more years is the life stage usually transported by world commerce. One of the main reasons that potato cyst nematode is of such importance economically is that at present there is no practical method to control it.

Root-knot nematodes damage both roots and tubers. One species, the northern root-knot nematode causes damage to potatoes in cooler areas while several other root-knot nematode species cause damage in warmer areas. Root-knot nematodes could possibly become a limiting factor in the production of potatoes in the lowland tropics or other warm climates of the world.

Root lesion nematodes also damage both roots and tubers. A number of species damage potatoes; some of which are adapted to cool climates and some to warmer climates. It is quite likely that root lesion nematodes, as well as other nematodes attacking potatoes, combine with certain fungi and possibly bacteria to cause disease complexes.

False root-knot nematode is recognized as a pathogen of potato in several areas of the Andes at elevations of from 3200-4200 meters (10,496-13,776 ft.). Observations of several workers indicate that false root-knot is a very serious potato disease at elevations where few food crops other than potatoes can be grown. Unfortunately, one of these crops, quinoa, a high protein cereal, is also susceptible to this nematode. The presence of both the potato cyst nematode and the false root-knot nematode in an area is a disaster for potato growers and those relying on potatoes grown in these areas for food.

### III. PRIORITIES FOR NEMATOTOLOGY RESEARCH

The nematology planning conference was held to examine priorities and recommend an action program of nematology research for the next five years. The following criteria were used to establish priorities.

1. Nematode distribution and present world-wide economic importance.
2. Predicted economic importance in relation to expanded geographic range of potato production.
3. International applicability of research results.
4. Probability of success of research projects.

The potato cyst nematode, root-knot nematodes, false root-knot nematode, and root lesion nematodes were considered at the conference. Based on the above criteria the following areas of needed research, listed in order of priority, were identified and recommended.

#### 1. Potato cyst nematode

Because of its widespread occurrence in most potato growing regions of the world, the potato cyst nematode (Heterodera rostochiensis and H. pallida) is at present the foremost important nematode pest of potato. Thus, potato cyst nematode should be given the highest priority in the nematology research program. Areas of research which would have the greatest impact on world potato production are listed below in order of priority.

- A. Breeding for resistance. Concentrate first on native cultivars and later on the wild species.

B. Pathotype identification. Concentrate on the populations occurring in the Andes of Peru, Bolivia, Ecuador, Colombia, and perhaps Argentina and Chile.

C. Search for tolerance. Field evaluations of native cultivars with inheritance studies on prospective tolerant lines.

## 2. Root-knot nematodes

The root-knot nematodes (Meloidogyne spp.) are widely distributed in the warm climates of the world. The successful adaptation of potato to warmer climates will undoubtedly increase the importance of root-knot nematodes as pathogens of potatoes. To minimize the effects of root-knot nematodes on potato production in warmer climates, the following areas of research are recommended.

A. Breeding for resistance. Concentrate on the native cultivars as sources of resistance and include wild species only if it becomes apparent no resistant genes are present in native cultivars.

B. Combining root-knot resistance with bacterial wilt resistance. Because of the positive interaction of these two pathogens on other solanaceous plants, interaction on potato is inevitable in climates where both pathogens exist.

## 3. False root-knot nematodes

The false root-knot nematode (Nacobbus spp.) is an extremely destructive pest of potato in the Andean region of Peru and Bolivia. At present its distribution and economic importance in other areas are not known. Very little is known about the false root-knot nematode, particularly as it relates to potatoes. The following areas of research are recommended to ascertain the importance of false root-knot nematodes to potato production in the developing world.

A. Species identification. Positive identification of species of Nacobbus spp. that attacks potato is imperative.

B. Life cycle and reproductive capacity. The reproductive potential of this nematode under various climatic conditions must be established.

C. Host range. Information is needed on the number and identity of plant species on which this nematode can survive.



D. Survival and dispersal. Because of important international implication, knowledge on how this nematode survives and spreads is of utmost importance.

E. Host resistance. On a limited basis, the native cultivars should be evaluated for resistance.

#### 4. Root lesion nematodes

At least nine species of root lesion nematodes (Pratylenchus spp.) attack potatoes. Since these species vary greatly in their soil and temperature requirements, root lesion nematodes represent a potential threat to potato production throughout the world. At the present, it is recommended that CIP not initiate a research program on root lesion nematodes. In the event root lesion nematodes become economically important internationally, CIP should be aware of current research on root lesion nematodes and possibly support such research through linkage projects. The areas of current research on root lesion nematodes which have the greatest international applicability are as follows:

A. Host resistance. Useful resistance to P. penetrans has been found in potato cyst nematode resistant varieties derived from S. tuberosum subsp. andigena.

B. Resistant cover crops. Certain cover crops resistant to P. penetrans have been identified for local areas. Others are needed that are adapted to others areas.

#### 5. Interaction of nematodes and other organisms

Since nematodes are, for the most part, debilitating parasites, they often predispose the plant to attack by other organisms or increase the severity of other diseases. Also, certain species of nematodes are known to transmit plant viruses. The potato cyst nematode, root-knot nematodes, and root-lesion nematodes are reported to increase the severity of Verticillium wilt of potato. There are no reported interactions involving the false root-knot nematode which is obviously due to the lack of extensive studies of this nematode.

Investigations of possible interactions of nematode and other organisms should be of lower priority research until more is known about the control of the major nematode pests of potatoes. However, obvious interactions should not be overlooked and the economic importance of such interactions should be determined in cooperation with scientists from

other disciplines at CIP. However, good judgment must be exercised to distinguish between possible interactions and simple associations.

#### IV. RECOMMENDED POTATO CYST NEMATODE RESEARCH

##### 1. Breeding for resistance

Developing a potato variety resistant to a plant pathogenic nematode is a long-time project requiring cooperation among nematologists, plant breeders, geneticists, horticulturists and often workers from other disciplines. Although, it may be the most economical control measure for growers, it is often the most expensive type of research. Genotypes with resistance to all pathotypes of H. rostochiensis and H. pallida occurring in the Andes probably would be resistant to pathotypes occurring in any other potato-producing area of the world. Thus results from this kind of research at CIP would be of great benefit to potato growers in all parts of the world where the potato cyst nematode occurs.

##### a. Present status of knowledge

Several sources of resistance to Heterodera rostochiensis have been found and good varieties which are resistant to this species are in the European and North American market. However, resistance to Heterodera pallida is as yet an unsolved problem. In Germany and Holland the approach has been to evaluate wild species for major genes conferring resistance to this nematode species. Their findings indicate that S. oplocense, S. multidissectum, S. sanctae-rosae, S. spegazzinii, and S. vernei are good species to test for genes which confer resistance to H. pallida. However, wild species require several backcrosses to make them acceptable by which time some of the resistant genes have been lost. In order to facilitate breeding, the approach in England has been to search for resistance to H. pallida in native cultivars of S. andigenum. They found three clones with resistance to H. pallida pathotype D and partial resistance to pathotype E.

The usefulness of resistant genes selected in Europe for nematode populations in the Andes is not clear. A preliminary test with differential plants on Andean populations shows that S. vernei line (VT<sup>n</sup>) 262.33.3 was resistant to eight populations, and S. kutzianum KTT.6021-19 was resistant to five populations from different areas of the Andes, whereas gene H<sub>1</sub>, H<sub>2</sub> and H<sub>1</sub>H<sub>2</sub> was resistant to only three populations, two of which were yellow. The action of gene H<sub>3</sub> has not yet been tested.

Breeding lines from other nematode resistant programs have not progressed sufficiently to be useful for resistance to the majority of Andean populations.

CIP's approach to this problem is dictated, in part, by the fact that there is a commitment to study the CIP germplasm collection which now contains 3-4 thousand native varieties; of which almost 2,000 have been screened against nematode populations from Huancayo (Chocon), Otuzco (Agrillpampa) and Cuzco (Ccotatoclla). This includes three years of field screening 1968-1971, and four pot tests, two at La Molina and two at Huancayo. However, wild species are also being tested for resistance.

The majority of clones selected for resistance from the CIP collection were S. juzepczukii,  $2n = 36$ . No clone of S. andigenum was resistant to the three nematode populations, although three clones were resistant to populations from Otuzco; 0041, 1422, and 1498.

b. Recommended program and approach

1. Although resistant genes in S. andigenum are scarce, screening should continue as genes can be combined and, if found, are of great value in breeding.

2. Screening of resistance in wild species should also be carried out as part of a long-term breeding program.

3. Screening at CIP, using test populations from Otuzco, Cuzco, and Huancayo should continue in order to detect specific genes for certain populations as well as to detect nonspecific resistant genes. Possibly a population from Ccotatoclla should be substituted for Andenes; both populations are from the Cuzco area.

4. Evidence from Germany and the USA suggests that some of the polygenes in wild species are lost during several backcrosses. This is an important area of research because the possibility of obtaining multigenic resistance in cultivars is dependent upon the transfer of polygenes from wild species.

5. It is proposed that certain aspects of screening for new genes for resistance to cyst nematode be done at locations other than CIP. This includes 1) screening commercial varieties for detecting minor genes for resistance to potato cyst nematode (England), 2) screening the German and Dutch Collections against H. pallida occurring in those

countries and possibly some of the Andean populations of H. pallida, and 3) studies of genes from resistant plants to understand their genetics and their homologies.

6. Promising resistant genes discovered at CIP or in national breeding programs should be distributed to interested investigators. Selections should be sent as tubers rather than as botanical seed to minimize problems of seedling testing, such as segregating populations and adaptation of the new seedling to the local environments. Seeds of  $F_1$  progeny should be sent only when quarantine regulations make it impossible to send tubers.

## 2. Classifying and determining distribution of pathotypes

The classification of pathotypes of the potato cyst nematode is of two fold importance: First, the Andean region is considered the area where the potato cyst nematode evolved and therefore one would expect to find in this region a different spectrum of pathotypes than those that are found in the potato growing areas of Europe and other countries. It is important to erect a pathotype scheme using a representative sample from the Andean region in order to understand the genetic variation of this nematode.

The second reason that classification is crucial is that it provides a rational basis for selecting a group of representative populations to use in screening for resistance, and at the same time to know the areas where these resistant genes will be useful in controlling the nematode:

### a. Present status of knowledge

Until 1972 populations of the potato cyst nematode which varied in aggressiveness were considered to be pathotypes of the same species, H. rostochiensis. Based on color of immature females, morphometric measurements of second stage larvae, and mating trials, a new species, H. pallida, was erected. Recent research has indicated that pathotypes exist in both species. The present knowledge of known pathotypes of both species is shown in Table 1.

Recent results at CIP indicate the presence of additional pathotypes which cannot be classified by the differential plants currently in use.

Classification of Andean nematode populations was started in 1972. Thirty populations were collected and are currently being subjected to tests devised in Europe to differentiate pathotypes.

Table 1. Current classification of known pathotypes of potato cyst nematode.

	Major resistance genes	<u>H. rostochiensis</u> yellow females				<u>H. pallida</u> Cream or white females		
		Nsr Mgs	Hrd Gbsh	Pr Hmz		Frsw		
German pathotype								
British pathotype		A				B	E	
Dutch pathotype		A	B	C	F		D	E
Susceptible potato	None	+	+	+	+	+	+	+
ex <u>andigena</u>	H <sub>1</sub>	-	+	+	-	+	+	+
ex <u>multidissectum</u>	H <sub>2</sub>	+	+	+	+	-	+	+
ex <u>andigena</u>	H <sub>3</sub>	+				-	-	
ex <u>andigena</u>	H <sub>1</sub> H <sub>3</sub>	-				-	-	
ex <u>and.</u> ex <u>mult.</u>	H <sub>1</sub> H <sub>2</sub>	-	+	+		-	+	+
ex <u>and.</u> ex <u>mult.</u>	H <sub>1</sub> H <sub>2</sub> H <sub>3</sub>	-				-	-	
ex <u>kurtzianum</u>	K <sub>1</sub> K <sub>2</sub>	-	-	+	+		+	+
ex <u>oplocense</u>	0	-					-	
ex <u>vernei</u> G-LKS58.1642/4		-	-	-	+		+	+
ex <u>vernei</u> (VTn) <sup>2</sup> 62-33-3		-	-	-	-		-	+

Female color showed that twenty-three populations were white (H. pallida), six populations showed a mixture of white and yellow females and one population appeared pure yellow (H. rostochiensis). All the yellow populations were found in the Puno and Arequipa areas of Peru.

When differential host plants were tested a wide variation in aggressiveness was evident. It is noteworthy that the yellow population from Arequipa behaved like pathotype A and that the population from Huancayo (Chocon) did not overcome the resistant genes of S. kurtzianum Ktt 6021-19 and S. vernei (VtN)<sup>262</sup>.33.3. This is the population that is being used for many screening tests and will be used for field testing. Three populations from northern Peru were very aggressive to every differential used.

Morphometric measurements of second stage larvae are underway. Previous data about populations from Huancayo, Cuzco and Otuzco show the variability of characters within each population to be much greater than the variation among European populations, but no significant or systematic differences were found.

Electrophoresis as a rapid method for differentiating between pathotypes or groups of pathotypes is being explored. On the basis of one experiment using immature females, the nematodes from Frampton (British E) were different from all the white populations from Peru and there was a similarity of banding patterns in nematodes from Otuzco with those from Puno.

b. Recommended program and approach

1. It is recommended that CIP continue research on identification, distribution, and classification of pathotypes of potato cyst nematodes in the Andes. It is suggested that further collections be made from Bolivia, Ecuador, Colombia, and perhaps Argentina and Chile to augment the collection from Peru. Studies of the collection should include: 1) reaction to differential hosts, 2) disk electrophoresis, 3) morphometric measurements, and 4) mating behavior.

The choice of differential host plants for screening Andean populations is very difficult. Obviously, identified wild species and cultivars with known differential value in the germ plasm collection at CIP should be used in addition to the differential plants used in Great Britain and Europe. In order to reduce the initial work load it is suggested that at first only test plants with known differ-

ential value be used and, furthermore, that only one clone of each of these cultivars and wild species be used.

2. It is suggested that some aspects of this work be carried out at locations other than CIP where facilities as well as trained personnel are available. For example, the work on chemical taxonomy, morphologic analysis, and mating experiments are in progress at Rothamsted. If future data indicate need for changes in specialization, this work should be conducted.
3. It is suggested that CIP develop a system of handling nematode populations to prevent the spread of aggressive populations into areas where they are not known to exist. CIP should insure that nematode testing areas at Huancaayo are protected by fencing and that facilities are available to destroy cysts in infested soil, plant material, and containers.
4. Scientists in Great Britain and Europe should lead in developing a uniform system of nomenclature for pathotypes of the potato cyst nematode. Preliminary tests at several locations already have been conducted. Scientists from CIP should cooperate in this endeavor in ways such as supplying nematode populations or differential plants and conducting experiments.
5. CIP should be supplied data, such as host ranges, concerning potato cyst nematode populations from various countries.
6. Differential plants should be exchanged among workers at CIP and national programs.
7. Because reproducible results and large numbers of tubers are needed, native cultivars rather than wild species should be used as differentials.

### 3. Search for tolerance

Tolerance to the potato cyst nematode is defined as the degree of endurance of the plant to the effect of infection of the root system by the nematodes. Damage is directly related to nematode population density in the soil. Below a certain density the plant is not sufficiently affected to result in a measureable reduction in yield. If potato varieties which are tolerant of high populations of nematodes can be found or developed, these could be grown in infested fields without danger of high yield losses. Use of such varieties should be limited to areas where the nematode is wide

spread because high nematode populations develop on tolerant varieties.

a. Present status of knowledge

Dutch workers have found tolerance to the potato cyst nematode in the cultivated variety "Multa". In field experiments where several potato varieties were badly damaged by potato cyst nematodes, "Multa" had a relatively better stand and higher yield than other varieties. Pot experiments showed that the initial density required to cause a 50% reduction in the weight of dry matter to be 1600 eggs/g of soil for "Multa" and 300 eggs/g of soil for "Libertas", a nontolerant variety.

In Peru 90 clones that had been selected by the national program because of good growth despite nematode attack were planted in 12 hill plots in a heavily infested field at Chocon. At harvest time (May 1973) 10 clones were selected which out-yielded Renacimiento by a threefold increase.

b. Recommended program and approach

1. It is recommended that CIP continue field evaluation of native varieties for tolerance to potato cyst nematode.
2. It is recommended that the inheritance of tolerance be investigated to determine its usefulness as background for breeding as well as for combining tolerance with resistance.
3. It is recommended that the possibility of developing a technique to determine tolerance by a pot method be investigated. A CIP staff member will be conducting research on this problem at Rothamsted.

c. Potential of other types of control

Plant resistance and/or tolerance offers considerable promise for practical control of the potato cyst nematode in Peru, in other countries of the Andes (Colombia, Bolivia, and Ecuador), and in many other potato-growing areas of the world where this nematode occurs. However, in most potato-growing areas it appears likely that several control measures (integrated control) may be needed to control this disease. Thus in planning a research program for CIP, control measures other than plant resistance must be considered.



## A. Chemical Control

### a. Present status of knowledge

Experiments concerning the effect of nematicides on populations of H. rostochiensis and on potato yields have been carried out for a number of years. Such tests have been conducted in Peru for approximately 10-12 years. In general, substantial yield increases and reductions of soil populations were obtained by the use of certain commercial and experimental nematicides. However, nematode populations in treated soil after the growth of only one crop of potatoes were usually as high or higher than before the nematicide was applied. Because of the need for yearly treatments, the relatively high dosage required for each treatment, and the high cost of nematicides, it appears unlikely that the use of current commercial nematicides will be practical except for only a limited number of growers in the Andes or, in fact, in most other potato-growing regions. At the present time, nematicides are used only to a limited extent as a control of potato cyst nematode by commercial potato growers.

Most of the recently-introduced and experimental nematicides are relatively nonvolatile and nonphytotoxic and some are systemic. Non-volatile nematicides can be applied with less expensive equipment than volatile ones and application by unskilled operators is possible. A disadvantage of some of the new nematicides is their relative high dermal and oral toxicity. Nematicides with low mammalian toxicity are needed.

Because systemic nematicides are used at relatively low dosages, especially those that can be applied to the foliage, they are more likely than non-systemic nematicides to be practical for controlling the potato cyst nematode. It is particularly important that a nematicide for use as a spray be relatively low in dermal and oral toxicity.

In addition to being nematicidal, some of the new nematicides also are effective insecticides. A treatment which would control one or more important insect pests of potato in addition to the potato cyst nematode is likely to be more practical than one that controls nematodes alone.

The use of a nematicide might be more practical if used together with other methods in an integrated control procedure. Crop rotation, varietal resistance or tolerance, and either the infrequent use of a nematicide at a high dosage or the frequent use at low dosages are possibilities.

b. Recommended program and approach

1. It is recommended that a research program involving the testing or adaptation of nematicides should not be conducted at CIP. Rather, it was suggested that a CIP staff member keep informed of nematicide research in national programs and commercial companies.
2. If such research develops an effective nematicide with systematic properties, nonphytotoxic to potatoes, and possessing low oral and dermal toxicity, CIP should then consider whether or not a research or outreach project on nematicides should be initiated.

B. Crop rotation.

a. Present status of knowledge

Because of the limited host range of the potato cyst nematode crop rotation probably has been the most widely-used control measure. Although rotation is effective, it is often impractical because of the length of rotation required when nematode population densities are high. Usually a long rotation consisting of only one year of potatoes in approximately seven years is needed to assure profitable potato yields if population densities are equal to, or above, plant damaging level. If densities are low and crop rotation is used to prevent them from reaching the damaging level, potato can be grown more frequently in rotation. A rotation in which potatoes were grown once every seven years has been used for centuries in the Andes. When land was owned by the community it was relatively easy to enforce these rotations. Such rotations are being followed today in Peru, particularly in mountainous areas where land is still controlled communally.

Crop rotation has been successful in preventing the build-up or in reducing populations density of potato cyst nematode in other potato-growing areas of the world. For example, 3-4 year rotations are used in England. Although potato growers experience crop losses with these relatively short rotations, short rotations are considered more practical than longer ones because of the lack of non-host crops which yield as much food or are as profitable as potatoes. In general, research data indicate that the change in soil population is approximately the same regardless of the non-host crop grown.

The numerous volunteer potato plants appearing in fields for several years following a potato crop decrease the effectiveness of crop rotation. This problem is more severe in potato-growing areas where winters are mild than in colder areas where soil temperatures are low enough to freeze those tubers remaining in the soil after harvest.

b. Recommended program and approach

1. It is recommended that a major research project involving crop rotation should not be initiated.
2. Because practical rotation crops and weeds vary in different potato-growing areas, rotation studies must be conducted in each potato-growing area where the potato cyst nematode occurs. Workers in national potato programs should be encouraged to test potential rotation crops and weeds for such factors as ability of root exudates to hatch larvae from roots and the potential of these exudates to kill the potato cyst nematode in the soil.

c. Biological Control

i. Present status of knowledge

Encysted larvae of the potato cyst nematode apparently damaged by fungi and bacteria have been observed frequently by a number of research workers. Other kinds of parasites and predators of this nematode have been noted. Because of the long association of this nematode with the potato in the Andes, natural enemies should be more abundant here than in any other potato-producing region.

ii. Recommended program and approach

1. The consensus was that a project on the use of parasites and predators as a control measure should not be initiated at CIP.
2. CIP should encourage studies in this area by visiting scientists and workers at other locations. For example, there is a possibility that a Rothamsted staff member will spend some time at CIP collecting and working with parasites of the potato cyst nematode. Collecting, isolating, and conducting pathogenicity studies with these isolates would make an excellent series of MS, Ph.D., post-doctoral, or sabbatical research problems for persons interested in and qualified to carry out this kind of research.

d. Cultural Control

i. Present status of knowledge

Various cultural practices often result in a reduction in the severity of the disease caused by the potato cyst nematodes. For example, complete disease escape by potatoes in infested soil does not occur, but partial disease escape by first-early varieties has been reported from Long Island, Scotland and Belgium.

These varieties start growth in spring at soil temperatures too low for much nematode activity, and may be harvested before many of the nematodes can reproduce. Thus these crops, because they escape serious damage and restrict multiplication of the pest, can be profitably grown frequently or continuously, on infested land. Crops of potatoes for canning, which also need a short growth period, similarly restrict nematode increase. In Northern Ireland two profitable canning crops were grown, one immediately after the other on the same infested (strain A) land in 1969. The overall increase in eelworm was 9-fold against 25-fold for a conventional ware crop. Disease escape was found to result from very late planting (August, instead of June or July) in Lithuania.

ii. Recommended program and approach

1. A research project in this area at CIP is not recommended.
2. While observing potato production practices in the Andes, CIP staff members should not overlook potential cultural control measures.

V. RECOMMENDED ROOT-KNOT NEMATODE RESEARCH

Root-knot nematodes (Meloidogyne spp.) are world wide in distribution but are limited to specific geographic areas by soil temperature and soil type. Root-knot nematodes are favored by warm soil temperatures, 25°C and above. However, certain species of root-knot nematodes have become established in somewhat cooler climates and cause severe damage in localized potato producing areas of the world.

Different species of Meloidogyne are important pathogens of potatoes in different countries. The most important species on potato in Europe and North America is M. hapla followed by M. incognita and M. incognita acrita. In Africa and Asia M. javanica and M. incognita are most important followed by M. incog-

nita acrita and M. hapla, the latter being found in Japan. M. arenaria has been found on potatoes in most continents.

Since potatoes are predominantly grown in the cooler climates of the world, root-knot nematodes are not at present a world-wide economic problem on potato. However, successful attempts to extend the range of potato culture into warmer climates such as the lowland tropics could drastically change this situation.

Other factors which increase the potential threat of root-knot nematodes to potato culture in the lowland tropics include: 1) the existence of a wide range which allows these nematodes to build up on many common weeds and rotation crops, 2) the rapid population increase (4-7 generations per season), 3) infection of tubers which permits easy transmission by seed, 4) the extreme susceptibility of potato to essentially all known species of Meloidogyne, and 5) Meoloidogyne spp. predispose numerous plants to attack by pathogenic fungi and bacteria. These facts illustrate the tremendous impact Meloidogyne spp. could have on potato culture outside its present range of major production.

# 1. Breeding for resistance

## a. Present status of knowledge

Limited work has been done on development of potato varieties resistant to Meloidogyne spp. Indian scientists have developed a potato variety (H-294) resistant to M. incognita. In addition, they found high resistance in Solanum vernei and S. spegazzinii and moderate resistance in S. bulbocastanum, S. gandailesii, S. lignicula, S. ajamhuiri and S. tuberosum subsp. andigena. In Peru, resistance to M. incognita in hybrids of S. demissum was found. Also, high resistance to Meloidogyne spp. in S. torvum and partial resistance in S. pseudalulo and S. quistoense has been reported. In the U. S. A., resistance to M. hapla, M. javanica, M. arenaria, and M. incognita acrita has been found in some clones (35 clones representing 15 families) in the S. tuberosum subsp. andigena population which is being selected for early maturity, yielding ability, and disease and insect resistance. Thus, resistance, to Meloidogyne spp. is apparently available in Solanum spp. and could be used in breeding programs.

## b. Recommended program and approach

1. It is recommended that a program be established at CIP to evaluate native Andean cultivars for resistance to Meloidogyne spp. Several species of Meloidogyne should be incorporated into the program as specific needs arise. It is further recommended that wild species of Solanum not be included in this program unless it becomes apparent

that there are no resistant genes in native varieties.

2. It is recommended that the root-knot nematode resistance program be closely coordinated with the program for resistance to bacterial wilt. Efforts should be made to combine root-knot nematode resistance with bacterial wilt resistance.
3. It is recommended that CIP cooperate with scientists from national programs in exchanging root-knot nematode resistant materials, particularly those programs where CIP linkage projects involving root-knot nematode resistance are in progress.
4. It is recommended that CIP not introduce species of Meloidogyne that are not present in Peru. Rather, it is recommended that screening for resistance to species of Meloidogyne not present in Peru be encouraged in national programs where the species is present.

2. Interaction with other pathogenic organisms

(See section on Interactions).

3. Potential of other types of control

A. Chemical control

a. Present status of knowledge

In some areas where Meloidogyne spp. cause serious plant damage, economic control has been achieved through the use of soil fumigants or the newer organic phosphate and oxime carbamate nematicides. Dosage levels depend upon soil type, environmental conditions, and type of crop.

b. Recommended program and approach

It is recommended that CIP not conduct a program on evaluation of nematicides for root-knot control. It is recommended, however, that CIP nematologists keep informed of current research in national programs concerning chemical control of root-knot nematodes. Such information would be helpful in advising outreach personnel in countries where serious root-knot problems might develop and resistance not be available.

## B. Crop rotation

### a. Present status of knowledge

Because of the wide host range of Meloidogyne spp. it is difficult to select suitable crops for rotation schemes. Workers in Rhodesia report that four years of weeping love grass, Katabora Rhodes grass, or Bambatsi Panicum grass in rotation with potato provided good control of M. javanica on potato. Perhaps rotation schemes developed for control of Meloidogyne spp. on other crops could be adapted to potato culture. Because of the relatively rapid decline of Meloidogyne spp. in the absence of suitable host, rotation schemes could be much shorter than that required for potato cyst nematode.

### b. Recommended program and approach

It is recommended that CIP nematologists not develop an active program on rotation for control of root-knot nematodes. However, it is recommended that where feasible CIP nematologists observe and record root-knot resistant crops that might be valuable in rotations with potato should a major problem develop and resistance not be available.

## VI. RECOMMENDED FALSE ROOT-KNOT NEMATODE RESEARCH

The "False Root-Knot Nematode" (Nacobbus spp.) is considered an important and sometimes the most important nematode parasite of potatoes in several Andean regions of Peru and Bolivia. In Peru it is distributed in the districts of Puno, Tacna and Moquegua. In the Central Sierra it occurs in Huancayo, Concepción and Jauja. It is found at altitudes of 3200-4200 meters (10,496-13,766 ft). The information available on the distribution of this nematode in the Andean and other potato growing areas of the world is not complete. There are at least two unpublished reports on the geographical distribution of this nematode in Peru, but, limited areas were surveyed and the information is somewhat redundant. Similarly, there is no information on the life cycle and behaviour of this nematode on potatoes. Since all the evidence indicates that this nematode is a very serious pest of potatoes in the Andes, a complete geographical survey and studies on the host-parasite relationships, life cycle and behaviour of this nematode in potatoes warrant attention.

1. Species identification

a. Present status of knowledge

Recent microscopic examination at CIP of specimens of Nacobbus sp. collected from Puno revealed that they are morphologically different than N. dorsalis and N. aberrans, the two valid species of this genus. Variations in the host range studies conducted at two different locations indicate the possible presence of different species or pathotypes of this nematode.

b. Recommended program and approach

It was agreed that the first priority in research on false root-knot nematode is to determine the species which attack potato. It is recommended that research in this area be continued at CIP and that populations from diverse areas be compared morphologically.

2. Life cycle and reproductive capacity

a. Present status of knowledge

The life cycle of Nacobbus spp. on potatoes has not been studied. Also, there are no data on the reproductive capacity of Nacobbus spp. on potato; however, field observations indicate that increase in population density is rapid.

b. Recommended program and approach

It is recommended that CIP conduct extensive investigations on the reproductive potential of Nacobbus spp. It is suggested that factors such as temperature, moisture, and soil type as they relate to reproduction be studied.

3. Host range

a. Present status of knowledge

There is limited information on the host range of Nacobbus spp. Of the few studies that have been made there are conflicting results. Surveys in Peru indicate that a large number of plant species are susceptible to Nacobbus spp.



b. Recommended program and approach

It is recommended that CIP conduct extensive investigations on the host range of Nacobbus spp. It is suggested that not only cultivated plants but also wild weeds in the area where this nematode is prevalent be studied.

4. Dispersal and survival

a. Present status of knowledge

There have been no studies on how the false root-knot nematode spreads within areas or between areas. Field observations indicated the possibility that seed plays an important role in the spread of this nematode. Also, the ability of a nematode to survive under adverse conditions is directly related to the number of ways it can be spread. There is no information available on the ability of Nacobbus spp. to survive adverse conditions or in the absence of a host.

b. Recommended program and approach

Since spread of Nacobbus spp. has important international implications, it is recommended that studies be made at CIP on its ability to infect or adhere to seed; its resistance to desiccation, and ability to survive in the absence of a host. Since the nematode causes severe damage in Peru and Bolivia, it is suggested that this work be done with the population that occurs in these areas.

5. Resistance and tolerance

a. Present status of knowledge

A limited study was conducted in Peru on resistance and tolerance in potato to Nacobbus spp. Resistance and/or tolerance was found in some clones of the native cultivars of ex-andigena.

b. Recommended program and approach

It is recommended that a program be developed at CIP to evaluate native cultivars for resistance and/or tolerance on a limited basis. It is suggested that this program not encompass wild species of Solanum at the present time.

6. Potential of various types of control

A. Chemical control

a. Present status of knowledge

There has been very little work on chemical control of false root-knot nematodes on potato. However, its similarity to root-knot nematode indicates that its response to chemical treatment would be similar to root-knot nematode.

b. Recommended program and approach

It is recommended that CIP not conduct evaluations of nematicides for control of Nacobbus spp. However, it is suggested that CIP nematologists keep informed of developments from national programs in this area of research and provide technical advice when the need is warranted.

B. Crop rotation

a. Present status of knowledge

At present no information is available on rotation as a means of controlling Nacobbus spp.

b. Recommended program and approach

Since there is limited information available on host range, it is proposed that CIP not study rotation but keep informed of developments in this area of research from national programs.

VII. RECOMMENDED ROOT LESION NEMATODE RESEARCH

At least nine species of lesion nematodes (Pratylenchus spp.) are reported to attack potatoes in various parts of the world. Thus, it appears that root lesion nematodes, although not at present as important economically as the potato cyst nematode or root-knot nematodes, are causing more loss to potatoes than is generally recognized. Both roots and tubers are damaged; root damage results in lowered yields and tuber damage in lowered quality. Soil temperature requirements vary greatly among species and apparently limit the geographical range of different species. Species with cool soil temperature requirements damage pota-

toes in such potato-growing areas as Europe and North America and species with warm soil temperature requirements occur in such areas as southern Japan and South Africa. Consequently, root lesion nematodes present a potential threat to potato production throughout the world.

1. Breeding for resistance

a. Present status of knowledge

Very little information concerning resistance of potato cultivars and selections to lesion nematodes has been published. Observations and preliminary data at Cornell University indicate that useful resistance to P. penetrans exists in potato cultivars and that even higher levels may exist within the germ plasm of tuber-bearing Solanum species. 'Peconic' and 'Hudson', potato cultivars with resistance to the potato cyst nematode derived from S. tuberosum subsp. andigena, are two of the cultivars with resistance to P. penetrans. 'Katahdin', the second most widely grown potato cultivar in the USA, is highly susceptible to this nematode.

b. Recommended program and approach

At the present it is recommended that CIP not initiate research on resistance to root lesion nematodes. Because root lesion nematodes represent a potential threat to potato production in many areas of the world, it is recommended that CIP nematologists keep informed of current research in this area currently in progress at other research centers. If root lesion becomes more of an international problem, then CIP could determine the advisability of initiating a program or supporting an existing program.

2. Resistant cover crops

a. Present status of knowledge

Crops such as rye, wheat, and millet commonly grown as cover crops or green manure crops in many potato producing areas favor extensive increase in the population density of P. penetrans. Certain cover crops such as oats and sudan grass are much less favorable for reproduction of P. penetrans and can be used in certain potato producing areas. Proper selection of cover crops can greatly reduce the preplant nematode population density and subsequent damage. To minimize the potential threat of root lesion nematodes to potato production, resistant cover crops which are adapted to other areas are needed.

b. Recommended program and approach

At the present it is recommended that CIP not initiate research to develop cover crops resistant to root lesion nematodes. It is recommended, however, that CIP nematologists keep informed of research in this area which is currently in progress at other research centers.

3. Potential of other types of control

A. Chemical control

a. Present status of knowledge

Although soil fumigation decreases populations of P. penetrans and increases potato yields, such a treatment is practical only when the acre value of a potato crop is relatively high, i. e. when yields and prices are relatively high. The profitable use of soil fumigants to control Pratylenchus spp. is further limited because usually soil fumigants are ineffective in fine-textured soils.

Some of the newer nematicides with lower vapor pressure and less phytotoxicity, such as aldicarb (Temik), Nematicur, Furadan, and Vydate are effective in a wide variety of soil types. Such nematicides are likely to be more practical than fumigants, because some of them can be applied at planting time without danger of plant damage, they are relatively easy to apply, and most of them kill insects as well as nematodes. At least one of them Vydate, is effective against Pratylenchus spp. when applied as a spray, which is an economical application method because potatoes must be sprayed to control insects and fungus diseases.

b. Recommended program and approach

At present it is not recommended that CIP conduct research on chemical control of root lesion nematodes. However, CIP nematologists should keep informed of developments in this area so as to properly advise outreach personnel should root lesion nematodes become a problem in certain regions.

B. Crop rotation

a. Present status of knowledge

Controlling root lesion nematodes by crop rotation is difficult because most species tested have a wide host range. However, crops such as marigold (Tagetes spp.) have been used successfully to reduce population density of certain species of root lesion nematodes. Crops are needed which are similar to marigold in effectiveness but which have greater agricultural value.

b. Recommended program and approach

It is recommended that CIP not initiate research on crop rotation to control root lesion nematodes.

VIII. RECOMMENDED RESEARCH ON NEMATODE INTERACTIONS

It is well established that nematodes interact with or transmit other disease-causing organisms including fungi, bacteria, and viruses. The most striking interactions are those in which plants bred for resistance to a particular disease are susceptible to the disease in the presence of nematodes. More recently, studies have shown that in many cases nematode infection changes the host physiology in such a way that organisms that are normally not pathogenic to a particular plant become pathogenic. Since most crop land is infested with plant nematodes, interactions are important in designing disease control programs.

1. Potato cyst nematode interactions

a. Present status of knowledge

Given suitable conditions, the potato cyst nematode and the black-scurf fungus Rhizoctonia solani together enhance disease and yield depression in potato. The potato cyst nematode also interacts with Verticillium dahliae, increasing the severity of the disease caused by the fungus. The interaction is characterized by earlier appearance of wilt symptoms and greater reduction in tuber yield than that caused by the fungus alone.

b. Recommended program and/or approach

It is recommended that studies of interactions involving the potato cyst nematode not be a major program at CIP. However, it is recommended that when striking interactions are evident, CIP nematologists cooperate with CIP scientists in other disciplines to determine the significance of such interaction. Good judgment must be

exercised to distinguish between possible interactions and simple associations.

## 2. Root knot nematode interactions

### a. Present status of knowledge

Interactions of root-knot nematodes with other disease organisms have been investigated more thoroughly than any other interactions. Consequently, root-knot nematodes have been implicated in more interactions than any other group of nematodes. However, there is only circumstantial evidence that root-knot nematodes are involved in interactions on potato, i. e., chemical control of M. hapla delayed the appearance of symptoms of Verticillium wilt. Important interactions of root-knot nematodes and disease of other solanaceous crops have been indentified. Root-knot nematodes have been found to alter the resistance and/or increase the severity of bacterial wilt of tomato and tobacco caused by Pseudomonas solanacearum. This suggests that if potatoes are grown in the presence of both of these organisms a similar interaction would exist.

### b. Recommended program and approach

It is recommended that studies of interactions involving root-knot nematodes not be a major project at CIP. However, because of the strong possibility that root-knot nematodes will interact with bacterial wilt of potatoes, it is recommended that efforts be made to combine root-knot nematodes and bacterial wilt resistance. Such combined resistance would minimize the problems likely to confront efforts to adapt the potato to the lowland tropics where both root-knot and bacterial wilt exist.

## 3. False root-knot nematode interactions

### a. Present status of knowledge

There have been no studies on the possible interaction of false root-knot nematode with other organisms. This does not eliminate the possibility that important interactions exist. The similarity of symptoms caused by this nematode and root-knot nematodes suggests the possibility that it may be as equally important in interactions as are root-knot nematodes.

b. Recommended program and approach

It is recommended that CIP not initiate a project on studies of interactions involving the false root-knot nematode. However, should obvious interaction be noted, CIP nematologists should investigate the importance of such interactions in cooperation with CIP scientists in other disciplines.

4. Root lesion nematode interactions

a. Present status of knowledge

The possibility that P. penetrans and other Pratylenchus spp. are associated with fungi such as Rhizoctonia solani, Verticillium spp., and Fusarium spp. in disease complexes makes difficult the interpretation of results of field tests designed to determine yield losses caused by Tratylenchus spp. Although it has been frequently mentioned in the published literature that such complexes occur there are few experimental data to support these statements. However, there is sufficient evidence to indicate that root lesion nematodes increases the severity of Verticillium wilt of mint, eggplant, and cotton.

b. Recommended program and approach

It is recommended that CIP not initiate a project to study interactions involving root lesion nematodes. However, CIP nematologists should be alert to possible interactions involving root lesion nematodes, particularly with Verticillium wilt, and investigate their importance when the need warrants.

5. Virus transmission

a. Present status of knowledge

Species of the genus Trichodorus are known to transmit tobacco rattle virus, the cause of stem "mottle" and tuber "spraing" or "corky ringspot" in potato. Other potato viruses suspected of being transmitted by nematodes include "tomato ringspot" and "tobacco ringspot".

b. Recommended program and approach

It is recommended that CIP not initiate a project to study virus transmission by nematodes. However, if nematode-transmission of important potato viruses is evident, CIP nematologists should investigate the importance of such transmission in cooperation with CIP virologists.

IX. RECOMMENDED FACILITIES FOR TESTING FOR  
RESISTANCE TO NEMATODES

1. Potato cyst nematode

The ability to accurately test the reaction of plants to specific populations of the potato cyst nematode is an absolute necessity in many areas of CIP's research on this nematode. The development of potato cyst nematode is temperature and moisture dependent and is influenced by soil type. Consequently, soil temperature must be maintained between 14 and 22°C, potted plants must be watered regularly, and a supply of suitable soil must be readily available.

Because of high soil temperatures, it is impossible to test for resistance to this nematode at La Molina, during the summer. Testing at La Molina in the winter is at best, precarious. During one winter the temperature of soil in pots on raised benches averaged 17°C and good results were obtained. However, during another winter when the average soil temperature was 24°C poor results were obtained and retesting was necessary.

To accommodate the resistance testing program at La Molina the following recommendations were made.

- a. A comparison should be made of soil temperatures in pots plunged into sand or soil with those in pots on raised benches. Perhaps when pots are plunged into sand or soil it will be possible to obtain consistently accurate readings every winter.
- b. At La Molina there should be an area with temperature control in which immature females and other developmental stages can be produced for use in specific research. If a suitable structure is sufficiently low cost, it could perhaps be expanded to accommodate



the resistance testing program during that part of the year when greenhouse temperatures are prohibitive.

At Huancayo it is questionable that accurate readings can be made even during the summer in a greenhouse without cooling. During the winter, heat would be required in a greenhouse. Excellent results have been obtained in cold frames in Huancayo during the summer months.

The following recommendations were made in regard to testing for resistance at Huancayo.

- a. To prevent loss of time, testing in cold frames should continue until adequate greenhouse facilities are built and tested.
- b. To guard against introduction and spread, nematode populations from parts of the Andes, other than Huancayo, should be used experimentally at the Santa Ana location only in greenhouses and cold frames surrounded by a fence. Also, drainage from these areas should not be in the direction of nearby potato fields. Some means, such as incineration or chemical treatment, should be devised to decontaminate soil that is discarded.

## 2. Root-knot nematodes

Because of different temperature requirements, testing for resistance to root-knot nematodes and potato cyst nematode cannot be conducted in the same facility at the same time. To accurately and efficiently test for resistance to root-knot nematodes, the soil temperature should be maintained between 24-30°C.

The following recommendations were made in regard to facilities for testing for resistance to root-knot nematodes.

- a. Because of distance and the inaccessibility during the rainy season of the root-knot infested field at San Ramón, it is recommended that initial screening for resistance not be done at San Ramón. Rather, the root-knot infested fields at San Ramón should be used for field performance of known resistance lines.
- b. It is recommended that adequate facilities be constructed at La Molina to accommodate testing for resistance to root-knot nematodes. This could be coordinated with the potato cyst nematode program. For example, temperature-controlled growth room could be used in the root-knot nematode program during the cooler parts of the year and for potato cyst nematode during the warmer parts of the year.

## APPENDIX I

### Position Paper on Nematology Research Planning Conference

B. B. Brodie and W. F. Mai

This paper was prepared as a result of a request from CIP to serve as a background for discussions during the Nematology Research Planning Conference at CIP in Lima, Peru, February 11-15, 1974. This is not intended to be a complete review of the status of research concerning the potato cyst nematode and the root-knot, false root-knot, and root lesion nematodes of potatoes. Instead the authors have attempted to include information relevant to topics to be discussed at the forthcoming conference.

Although the names of authors responsible for some statements and concepts are included in the text, all data presented are not supported by authors' names. A list of citations of publications is not included. However, complete citations of the articles referred to in the text, and related publications will be brought to the conference. In addition, when possible, abstracts and reprints of these articles will be available for use at the conference.

In this paper the term "vertical resistance" is used to refer to resistance to development of a nematode which is due to one or two major genes; "Horizontal resistance" or "field resistance" refers to resistance due to polygenes, and "tolerance" refers to the ability of a host species or cultivar to yield satisfactorily despite the presence of relatively high numbers of a pathogenic nematode. The species H. pallida is used only in connection with data published after this species was described in 1972. In discussing information published prior to this date the British and Dutch pathotypes with white or cream-colored females are considered as H. rostochiensis.

In accordance with a suggestion from a participant at the CIP late blight planning conference, one scientist scheduled to attend the nematology conference has been invited to serve as the discussion leader for each of several topics to be considered.

Suggestions made concerning research objectives for a program at CIP should be considered as tentative and are included only to stimulate discussion.

**6**

***Cold Hardiness***

## CONTENTS

	<u>Page</u>
I      Agenda	246
II     Introduction	249
III    Physical and Chemical Factors Relating to Cold Hardiness	249
IV    Sequential Testing Procedures	252
V     Recommendations - Preamble	257
VI    Recommendations	262
APPENDIX I	264
APPENDIX II	300

The participants attending the Planning Conference were as follows:

C. J. Weiser

Head, Department of Horticulture  
Oregon State University  
Corwallis, Oregon 97331  
U. S. A. -

L. V. Gusta

Crop Science Department  
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Saskatoon, Sask. S7N 0W0  
CANADA. -

M. J. Burke

Laboratory of Plant Hardiness  
Department of Horticultural Science  
University of Minnesota  
St. Paul, Minn. 55101  
U. S. A. -

It is with regret that two invited participants were unable to attend:

Prof. D. Fenson, Head, Department of Biology, Mount Allison University, Sackville, New Brunswick, Canada; Dr. Krasavtsev, Timiriazev Institute of Plant Physiology, Botanicheskaya Street, Moscow 127273, U. S. S. R.

CIP personnel in attending at the Conference were:

K. D. Sayre	Head, Department of Physiology CIP, Lima
P. H. Li	On sabbatical leave from the Laboratory of Plant Hardiness Department of Horticultural Science- University of Minnesota
N. Estrada	Department of Breeding and Genetics CIP, Lima
W. M. Roca	Department of Physiology CIP, Lima
F. N. Ezeta	Département of Physiology CIP, Lima
O. T. Page	Director of Research CIP, Lima

A position paper entitled: "A Review of Potato and Herbaceous Plant Hardiness" compiled for the Conference by Drs. L. V. Gusta and M. J. Burke is appended (Appendix 1).

CENTRO INTERNACIONAL DE LA PAPA

COLD RESISTANCE PROJECT PLANNING CONFERENCE

I. AGENDA

Monday, February 25

- 9:15 Introduction of Participants
- Overview of Objectives of the Planning Conference  
Dr. R. L. Sawyer, Director General
- 9:45 General Comments on Position Paper  
Drs. M. J. Burke and L. V. Gusta
- 10:00 Coffee
- 10:15 I Biochemical Considerations of Frost Tolerance  
Dr. L. V. Gusta
- a) Membranes
  - b) Protein; nucleic acids
  - c) Carbohydrates
  - d) Plant and growth substances
- 12:00 Lunch at La Molina.

Monday, Afternoon

- 1:30 II Biophysical Considerations of Frost Tolerance -  
Dr. M. J. Burke
- 3:00 Coffee
- 3:15 - 4:15 III Theories to Explain Frost Tolerance -  
Drs. Burke, Gusta
- a) The sulfhydryl hypothesis
  - b) The second supercooling point hypothesis
  - c) The vital water exotherm hypothesis

Tuesday, February 26

- 8:45 IV Breeding for Frost Tolerance - Dr. N. Estrada
- 9:15 Discussions of Breeding Approach
- 10:00 Coffee
- 10:15 V Methods of Determining Frost Tolerance -  
Dr. C.J. Weiser
- A. Biological Methods
1. Whole plant tests
  2. Cut leaf tests
  3. Viability tests
    - a) Neutral red
    - b) Conductivity method
    - c) Plasmolysis technique
    - d) Triphenyl tetrazolium chloride test
- 12:00 Lunch at La Molina
- 1:30 Methods of Determining Frost Tolerance - continued
- B. Physical Methods
1. Nuclear magnetic resonance
  2. Thermal analysis
  3. Electric resistance
- 3:00 Coffee
- 3:15 - 5:00 Preparation of Summary Report
- 7:30 Completion of Summary Report

Wednesday, February 27

- 9:00 Discussion and Recommendation of Methods of Determining Frost Tolerance Applicable to CIP
- A. Biological Methods
1. Whole plant tests
  2. Cut leaf tests
  3. Viability tests



10:15            Coffee

10:30            B. Physical Methods

12:15            Lunch at La Molina

1:30             Discussion of Breeding Program and Recommendations

                 A. Definition of Objectives

                 B. Selection of clonal material

                 C. Facilities for testing frost tolerance

3:15             Coffee

3:30 - 4:30      General Discussion - CIP Physiology Program

                 Dr. K. D. Sayre

## II. INTRODUCTION

Environmental factors such as excesses of cold, solar radiation, soil dryness and soil wetness, relative to the potato plant, may result in stress responses which reduce yield and quality. While developing countries may have areas in which one or several environmental factors may cause stress during a growing season, it was the intent of the Planning Conference to concentrate on responses of the potato plant to temperature in the range, 0 to -6 C. By improving cold hardiness it is anticipated that potatoes can be grown at higher altitudes thus expanding the areas in which potatoes can be profitably produced in Andean countries, Kenya and India.

Freezing temperatures might occur only at the beginning and end of a growing season, or they may occur sporadically throughout a season. In the former case varieties of potatoes which mature in 90 - 110 days may escape frosts. In the latter case, maximum cold hardiness obtained through diligent breeding offers the best hope for either increased resistance to cold or recovery from frost injury.

While chemically induced cold resistance is a future target, injury from airdrainage frosts and risk of injury from rapid thawing of frozen plants may be minimized through careful site selection using present technology.

## III. PHYSICAL AND CHEMICAL FACTORS RELATING TO COLD HARDINESS

It was generally agreed that Solanum species do not acclimate to low temperatures. Their ability to withstand cold is an inherent ability which does not change markedly during the growing season. Although there may be biochemical markers of frost tolerance, such as enzymes, direct determination of the frost killing temperature is considered the favored approach. The range of cold hardiness for the Solanum species discussed was between -2°C and -6°C. Both S. tuberosum and S. acaule are truly frost tolerant and can withstand freezing of as much as 50% of the tissue water without serious injury. Supercooling in potato plants was not considered an effective means of frost hardiness. This tolerance to freezing was discussed in terms of the tissue membranes, proteins, carbohydrates, etc. As is pointed out in the background paper these substances have been implicated in frost tolerance in other plants.

- A. Membranes: As many plants acclimate to low temperature, increases in the degree of unsaturation in fatty acids become apparent. This may be important for cold tolerance because an increase in unsaturation makes the membranes more fluid, reduces the probability of a lipid phase transition and makes the membranes more permeable to water.
- B. Proteins and carbohydrates: Changes in both of these substances also occur when plants acclimate to low temperatures. Their involvement in frost tolerance is less well understood than membranes. In the case of proteins their importance seems to revolve about their ability to withstand denaturation in the condensed protoplasm of a frozen tissue. Factors of importance here are stability to changes of pH, ionic strength, close association, etc. Low molecular weight carbohydrates may be of importance in protecting proteins and membranes, in controlling the osmotic potential of the cell and in altering the growth patterns of ice as described by Olien. In cases where plants do acclimate to low temperature, the timing of carbohydrate alterations does not correspond directly to the onset of cold acclimation, and therefore, this alteration alone does not appear to be the triggering mechanism for cold hardiness. Plant growth substances are important in the timing of cold acclimation in plants which can acclimate to low temperature; however, potatoes do not seem to be able to undergo cold acclimation.

Various theories have been developed to explain cold hardiness. Three were discussed: the sulfhydryl hypothesis of Levitt, the second supercooling point hypothesis of Tumanov and Krasavtsev and the vital water exotherm hypothesis of Weiser. All three of these hypotheses deal with the problems arising from protoplasmic dehydration and are discussed in the background paper.

As a cell is frozen first extracellular ice is formed. Continued freezing of water must involve intracellular freezing which is always fatal to the cell or removal of liquid water from the cell which is then frozen in the extracellular region. This latter extracellular freezing is not always injurious to plant tissue and in fact is responsible for survival in hardy plants. The sulfhydryl hypothesis recognizes the importance of cell dehydration as a mechanism of avoiding intracellular ice. The sulfhydryl hypothesis also recognizes the harmful effects of cell dehydration. The condensed protoplasm will probably have altered ionic strength, pH, close macromolecular contacts, and at the maximum, the remaining unfrozen water will be in thin films no thicker than several monolayers. Levitt suggested that the above conditions are conducive to the oxidation of sulfhydryl groups on adjacent macromolecules to form disulfide bridges, cross-linking and irreversible

denaturation of macromolecules. The fact that the presence of reducing agents which inhibit disulfide bridge formation prevents cellular injury by frost supports this hypothesis. However, disulfide bridges formation need not be the only source of protein cross-linking and denaturation. Hydrogen bonding between adjacent proteins can lead to similar results and frequently even the most careful protein precipitations are irreversible processes when protein cross-linking by disulfide bridges and hydrogen bonds are not involved. The major point to be obtained from the sulfhydryl hypothesis is that in the condensed protoplasm of frozen tissues macromolecular aggregation is the major factor leading to injury.

The vital water exotherm hypothesis and the second supercooling point hypothesis were both proposed to explain low temperature exothermic events observed at the killing point of woody tissues. Although such exotherms are not observed in Solanum species, the considerations leading to the proposal of these two hypothesis are relevant in the discussion of potato frost tolerance.

The vital water exotherm hypothesis recognizes the importance of water in the maintenance of macromolecular structure. Protein crystals themselves often contain more than 50% water and removal of the water destroys the crystals and often to the denaturation of the component proteins. In frozen tissue there is a competition between extracellular ice and intracellular substances for liquid water. These substances include proteins. At the killing temperature the water necessary for macromolecular stability is removed from the cell leading to the irreversible denaturation of the unstable macromolecules. This hypothesis can accomodate, but does not require macromolecular aggregation.

The second supercooling point hypothesis suggests that at some point during the freezing of a tissue the movement of liquid water from the cell becomes restricted due to a sudden change in the membrane permeability. The intracellular water there supercools to below its freezing point before freezing; the intracellular freezing kills the cell. These hypotheses all involve the transport of water from the cell during extracellular freezing and are not mutually exclusive.

Factors only peripherally related to frost hardness may be of importance in reducing yield losses. Two considerations which surfaced were the development of early maturing potato varieties for the Andean region which could be grown in periods of low frost probability. Regrowth ability in frost-injured plants could also be of considerable importance in reducing yield losses resulting from frosts.

Preliminary results on the freezing process in S. acaule and S. tuberosum indicate that approximately 50% of the leaf water is frozen at the killing temperature. In S. acaule 50% of the leaf-water was frozen at  $-5.5^{\circ}\text{C}$  and in S. tuberosum 50% of the tissue water froze at  $-2.5^{\circ}\text{C}$ . Other Solanum species

being investigated are S. multidissectum, S. chomatophilum, S. bukasovii and S. commersonii. Winter wheats are killed when 87% of their leaf water is frozen (Appendix 1).

Rapid methods for screening the frost hardiness of plants have been the limiting factor in breeding for resistance. Top priority must be given to rapid screening techniques. Screening techniques are of two general categories, whole plant tests and cut leaf tests. The survival of the whole plant in the field is the final desired result. A limitation of the whole plant test in the field is the absence of climate control. Whole plant tests in the growth chamber provide temperature control, but some care must be exercised to make sure there is a uniform temperature in the freezing chamber and that roots and other plant parts are not subjected to the cold stress. Correlation between the cut leaf killing temperature and the whole plant killing temperature is very good. An advantage in the cut leaf test is that the plants do not have to be sacrificed and can be used for other tests.

#### IV. SEQUENTIAL TESTING PROCEDURES

Laboratory screening techniques for frost resistance must consider the different types of frost which occur in the field. The two major types of frosts which may occur are from air drainage and radiation frosts. Other environmental factors which may influence the degree of injury are moisture content of the soil and the relative humidity of the air.

Field tests should be designed to take in the above factors and the results obtained in the field compared with artificial freeze tests. Good relative agreement of artificial freeze tests with field tests is essential. Knowledge of the factors which influence survival in the field will permit tailoring of the artificial freeze tests. These tests should be limited in that they ensure validity of the artificial freeze tests.

##### A. Field survival tests:

1. The influence of air drainage. Test sites could be located on the side of a hill with a gentle slope. Genotypes with a known range of frost tolerance would be planted along the hill. Since the lowest part of the hill would be the coldest a gradient effect would be created due to air drainage. Temperature recording devices should be used to monitor the temperature at different elevations. Following a frost the plants would be evaluated for

injury. Results from this study would then be compared to artificial freeze tests.

2. Radiation frosts. Radiation frosts occur on clear cold nights where there is little or no movement of air. This results in conditions where the temperature of the leaves is lower than the surrounding air. Test sites should be selected at the higher elevations where conditions would favor this occurrence. Replicated trials with genotypes of known hardiness would be planted out. Following a radiation frost the plants would be rated for survival and the results compared to artificial freeze tests. Ideally it would be of benefit to know both, the leaf and air temperature during a frost.
3. East vs. west slope planting. There is evidence to indicate that potatoes planted on the east slope of a hill are injured to a greater degree following a frost than potatoes located on the west slope. This is thought to be due to the rapid thawing of frozen plants by the morning sun. The rate of thawing may be then another factor in addition to low temperature that affects survival and should be considered in the artificial freeze. A series of shading experiments for plants located on the east side of a hill would help resolve this question.
4. The influence of maturity. There is little evidence on whether maturity has an effect on the hardiness of potatoes. A date of planting experiment could be initiated in an area where frosts are predictable for a given month. Genotypes of a known range of hardiness could be planted every second or third week up to the time when a frost usually occurs. This would establish a series of plants at different stages of maturity. Following a frost the plant would then be rated for injury.

In all of the above field test temperature recording devices should be located at the test sites to provide information on the minimum temperature, the duration of the minimum temperature, and the rate of temperature change.

5. Collection of hardy genotypes. A survey of farmer's fields in the high altitude areas following severe frosts may be one means of selecting very hardy genotypes. Many of these potato fields do not consist of pure lines and also some of the potatoes are from very old lines. There may be good potential of frost hardiness in these older lines since they have been handed down from generation to generation. Genotypes which clearly show superior hardiness should be collected and tested for cold hardiness by

artificial freeze tests at the Center.

B. Artificial freeze tests:

Artificial freeze tests have the advantage of precision in temperature control, flexibility in regard to the desired temperature and the experiments may be replicated in time. A standard freeze test would permit comparison of results obtained by different researchers.

1. Immediate considerations. A low temperature water bath is available at the Center now which would permit immediate screening of genotypes. Sukumaran has developed an artificial screening technique using the cut-leaf test for potatoes. One test temperature e. g.  $-4^{\circ}\text{C}$  would be suitable initially for screening populations and quickly removing the non-hardy genotypes. Plants could be nucleated at  $-1.5^{\circ}\text{C}$  and rated for damage either visually or by conductivities. Modifications should be carried out to shorten the time required for the freeze test. Care should be given to the avoidance of samples drying out during preparation.

2. Short range considerations.

a) Whole plant freeze tests. A freezing test chamber will be available at the Center shortly which would be suitable for freezing whole plant either in flats or in pots. Dr. N. Estrada is familiar with this technique and has published on it. The freezing test chamber could be used to compare the results obtained from the cut leaf tests. There is evidence that there is agreement between the cut leaf test and whole plant test. In the whole plant test the complete plant may be sacrificed; however, if the plants are generated from cut seed pieces the breeding stock may not necessarily be lost. Seedling stock, however, may be lost. This chamber may also be used to evaluate regrowth following a freezing stress. This information would be of value when plants are exposed to injurious frosts early in their development.

The whole plant freeze test may also be used to determine the effect of repeated frosts on survival. Plants which survive a single frost of  $-4^{\circ}\text{C}$  may be injured by a subsequent frost of  $-2$  to  $-3^{\circ}\text{C}$ . Repeated frosts in the field are not uncommon and their effects should be considered in artificial freeze tests.

The whole - plant freeze test could be used to determine if

compounds are translocated from injured leaves to the remaining plant parts which have an effect on subsequent effect on regrowth and recovery.

An extension of the program would be the incorporation of a modified domestic deep-freeze. The conversion of domestic deep freezers has been described in the Canadian Journal of Plant Science. This type of freezer offers a finer control over temperature as compared to commercial growth chamber. In addition to using it for whole plants it may also be modified for use with the cut leaf test. A fan would be installed in the test chamber for maintaining a uniform temperature.

- b) Temperature gradient bar. The construction of a temperature gradient bar would facilitate the screening of large populations. This method would be of value in determining the hardiness of genotypes within one degree. Other experiments of value which could be done are to determine the effect of frost duration on survival and the effect of the rate of temperature change on survival.
- c) Viability tests. At present the simplest test for evaluating frost injury is the visual test. It is rapid and cheap and agrees with field observations. Other methods used for estimating injury are laborious, slow and costly. The conductivity method is relatively simple and quick and provides a good objective test for rating injury.
- d) Frost hardiness rating of climate races. Within a given race there may be considerable differences in frost hardiness. A good example of this is S. acaule which has been reported in the literature to withstand a range of temperatures from -4 to -9°C. Knowledge of the variation on hardy genotypes would be essential in a breeding program and may also be of benefit in elucidating the mechanism of frost hardiness. The most cold hardy races would be the material of choice in breeding programs. Climatic races should be collected from a range of habitats and then evaluated for cold hardiness by artificial freeze tests. A series of test temperatures should be employed to determine the hardiness within one degree. Once the hardiness has been assessed the plants could be maintained in the germ plasm collection for future reference.



- e) Reference table for frost hardiness. Since the Center has the best collection of potato germ plasm in the world a reference table could be established for frost hardiness. Genotypes could be screened for hardiness under controlled conditions and then ranked in order of hardiness. This information would be of considerable value for plant breeders around the world which have a frost problem with potatoes.
- f) Effect of moisture on cold hardiness. The water status of the plant has been shown to have an effect on cold hardiness. In irrigated areas a study may be initiated to withhold water during critical cold periods to determine the effect on cold survival. Follow up tests could also be conducted in the laboratory.

#### Long Term Considerations

The ultimate screening technique in screening for frost tolerance should be simple, quick and non-destructive. Although such a method does not exist at present perhaps as we gain more knowledge on the freezing process the ultimate test will be developed.

With the establishment of a breeding program for frost hardiness other factors such as photoperiodic insensitivity (day neutral with regard to tuberization) disease and insect resistance may have to be evaluated to determine how these are inherited along with frost hardiness. The determination of the mode of frost hardiness inheritance could greatly facilitate an effective breeding program. It is still not known if hardiness is quantitatively or qualitatively inherited, whether maternal inheritance is a significant factor, or whether extensive epistasis offers potential for telescoping breeding cycles. Resolution of these and related genetic questions could increase the effectiveness of breeding efforts and shorten the time required to develop frost resistant cultivars with the desirable characteristics.

There is some evidence to indicate the number of stomata may be involved in leaf frost hardiness. Once this observation has been established the mechanism should be studied if there is a positive correlation.

## V. RECOMMENDATIONS - PREAMBLE

### A. Considerations in arriving at recommendations:

1. What is needed to avoid or significantly attenuate losses due to frost injury in potato? (an idealized projection of objectives).
2. What roles can and/or should the Center play in meeting these objectives? (unique strengths of the Centers and capabilities for providing international leadership and coordination to such a program in ways which are commensurate with overall charge and objectives of the Center).
3. What can likely be achieved in reducing freezing damage and losses to potato. (Realistic assessment and identification of priorities for immediate (1-2 years); short range (3-5) and long range (5-10 years) goals).

In making recommendations the conferees have attempted to take into account the considerations listed above. Insofar as possible the planning process involved sequential consideration of these considerations in the order they are presented; e. g. idealistically what would be the ultimate solution (s) -- What are the unique capabilities of the Center in achieving such solutions? -- Why is it realistic to undertake and in what order?

Since time, resources, and manpower are limited for addressing all problems associated with attenuating frost damage to potatoes, (and other important problems) it is necessary to make certain assumptions in arriving at recommendation -- particularly in focusing effort in the immediate and short-range phases of the program. These assumptions are listed in the next section.

As work progresses, and these assumptions are verified or proved to be invalid, it will be appropriate and necessary to alter the priorities and recommendations accordingly.

### B. Assumptions made in arriving at recommendations.

- I. Frost damage is a major limiting factor to potato production on a worldwide basis; a problem of sufficient magnitude, scope and impact to warrant a significant effort on the part of CIP.

2. The problem of reducing frost losses on a field scale can be approached in several ways, but breeding frost resistant cultivars provides the best means of achieving that end. (Appendix B).
  3. Screening for frost resistance is a relatively simple process compared to screening for other pest, disease, and stresses which limit production.
  4. Screening methods now available provide valid means of selecting genotypes which will have field resistance to natural frosts. a) The relative hardness of excised leaflets reflects accurately the relative resistance of whole plants in the field; b) Avoidance of freezing by supercooling is not a significant factor in frost avoidance under field conditions; c) Potato plants do not acclimate significantly to resist freezing stress; d) The relative hardness of seedlings reflects the relative hardness of mature plants grown from tuber seed pieces; e) Relative differences in resistance of young and old leaves to frost are similar among genotypes; f) The nature of freezing resistance in different potato species and genotypes is basically similar and related primarily to the status amount of unfrozen water in the tissues; g) Leaves are the most frequently injured tissues and appropriate test tissues for assessing field hardness; h) Freezing resistance is closely related with thawing resistance which may be a factor in field damage; i) Repeated exposure to frost is likely to accentuate (amplify) injury.
  5. Efficient mass selection procedures can be developed and refined within the next three years which will permit efficient screening of large populations.
  6. Further elucidation of the nature of freezing stress in the field and the nature of the freezing process in potato leaves is appropriate, and offers the most likely avenue for long-ranged break-throughs in genetic improvement and/or physiological manipulation of potato to increase resistance and reduce damage caused by freezing and perhaps other stresses (drought, heat, salt) which also involve tissue dessication.
- C. Narrative and Elaboration on Considerations and Assumptions itemized in A and B.
1. Productive cultivars of potato which resisted  $-6^{\circ}\text{C}$  at all stages of development would solve most of the frost problems which now limit production in many parts of the world. The impact of frost

resistant cultivar development would be considerably accentuated if frost resistance was incorporated into genotypes which also have a wide range adaptation -- especially in terms of photo periodic insensitivity in tuber initiation. Attempts to genetically achieve a combination for these characteristics would be a worthwhile long-range objective.

2. Although a number of wild species of tuber-bearing *Solanums* possess frost resistance of the magnitude mentioned in the preceding section, there has been little progress in incorporating this characteristic into cultivated varieties. The Center is in a unique position to provide leadership and coordination to the development of frost resistant potato cultivars resistant for several reasons involving personnel, geographic and germplasm considerations. There are no insurmountable or even major barriers to progress. As described in another section of this report on breeding implications, crossability, heritability, sources of resistance, and other genetic considerations do not limit progress. Valid controlled freezing tests are now also available which, with minor refinements, can be adapted for mass selection.

The missing element which has limited progress in breeding frost resistant potato varieties has been and is a team approach combining breeding and physiological expertise into an effective working unit. Geneticists and pathologists have worked effectively together in breeding for disease resistance, but cryobiology and stress physiology, is a specialized field of study which few breeders feel competent to undertake and where few research colleagues have been available to cooperate directly. The critical mass of interdisciplinary expertise provided by the Center staff offers a unique opportunity to overcome this deficiency which has seriously hampered previous programs. Clearly the unpredictability and variability implicit in "test freeze" field selection for frost resistance have made that approach an inappropriate and unproductive way to achieve and sustain an effective mass selection program.

While mass selection for frost resistance in the field has not been effective there are a number of important questions that can only be resolved in the field. The germplasm collection, proximity to highland production areas where frost is a serious problem in the field, access to native species in their natural habitats, access to field testing sites subject to frost and other geographical considerations, provide the Center with unique opportunities to effectively evaluate assumptions (Particularly those listed in Section B) item 4) and explore potentials. Appropriate studies which take advantage of germplasm and geographic features of the Center operation

are described in more detail in the recommendations section of this report.

3. Frost problems and opportunities for solving them take several forms. Emphasis in this report are placed heavily on breeding for resistance because the conferees believe that this offers the best chance for significant reduction of losses from freezing in areas where frost is a crucial problem. Physiological and cultural manipulations to reduce frost damage may also arise which warrant attention, but none look particularly promising at this time. In irrigated areas withholding water prior to frost may be worth exploring since highly hydrated tissues are more subject to freezing damage in some crops. Heavy potassium fertilization seems to increase frost resistance in potato, but the practical implications are doubtful. Considerable chemical cryoprotectant work is underway on a variety of crops but to date there has been no striking success and no widespread commercial use of chemical protectants. It would be well to keep a close watch on developments on other crops, but undertaking extensive efforts on potato seem ill-advised unless something promising is identified. Frost problems and possible approaches differ somewhat in different situations; e. g. In:

- a) High elevations where frost cause extensive losses; where frosts can and do occur any month of the year; and where potato is a dietary staple in the farmer's diet.
- b) In commercial production areas where frosts at either end of the generally frost-free growing period cause significant losses.
- c) In new areas where potatoes are not now grown.

The immediate and short-term recommendations in this report are aimed primarily at situation a) in the preceeding list because frost losses in these situations constitute the most recurrent and serious losses in terms of their social, if not economic, impact. Situation b) probably warrants a different approach- specifically selection for early maturing types which can avoid damage by maturing during the frost-free growing season rather than breeding for resistance per se.

- D. 1. There is little data available on the world-wide losses caused by frost on potatoes although it has been said to be the single most limiting factor to production in many areas. This lack of

definitive information may be due in part to the acceptance of crop losses from uncontrollable natural causes such as weather. At the high elevations in India, Kenya, Colombia, Ecuador, Bolivia, Peru and elsewhere where frost is a particularly serious problem, much of the production is for home consumption, and definitive data on production and losses due to frost are much more difficult to collect than in commercial production areas.

Several points, however, are clear. Fall and spring frost damage is almost a ubiquitous problem wherever potatoes are grown in the temperate zones. In the mountain and high plateau areas of Africa, South America, and the Indian sub-continent where the native people are most dependent on potatoes as a major dietary staple food the problem of frost damage is unfortunately most severe. (See "Prospects for the Potato in the Developing World", pages 27-44, 50-53, 217-224).

2. The major points are: Potato leaves frozen under controlled conditions either survive or are killed; that visual observation of the water-soaked appearance of dead leaves or simple conductivity tests can be used to evaluate injury; there appears to be good correlations between controlled freezing tests and field survival. Compared to screening for other types of pest or disease resistance this is an extremely simple, straight forward procedure. Because of this it seems reasonable to tool up for screening of large populations for frost resistance. Where concurrent breeding programs are undertaken (e. g. for development of frost and nematode resistant, day-neutral cultivars) it would be reasonable to screen initially for frost resistance, and to screen the much smaller populations remaining for other characteristics which aren't so readily evaluated.
3. The preliminary NMR data which indicates that death in frost resistant frost susceptible genotypes may occur at the point when 50% of the water is frozen (50% of the water is frozen at different temperatures) suggests that there may be some unifying principles involved in frost injury and resistance in potato. Elucidation of these principles could provide basic information with far-reaching implications for increasing frost, drought, salt and heat resistance of potatoes and other crops; stresses which all involve resistance, avoidance, or tolerance of protoplasmic dehydration. Frost resistance research on potato could also point the way to similar research on other herbaceous crops which have received little if any attention (e. g. corn).

## VI. RECOMMENDATIONS

### I. SHORT TERM (1 - 3 years)

1. It is recommended to establish whether the relative hardness of excised leaflets reflects accurately the relative frost hardness of whole plants in the field:
  - a) To verify that significant acclimation does not occur so as to invalidate relative cold hardness ratings;
  - b) To verify whether the cold hardness of excised leaflets of varying physiological age reflects the hardness of whole plants under controlled conditions;
  - c) To determine whether a correlation exists in the cold hardness of selected genotypes (with known hardness range) between Laboratory and field hardness responses.
2. It is recommended to utilize available equipment resources to commence excised leaflet screening tests as soon as possible.
3. It is recommended that equipment such as (I) a thermal gradient bar be developed and perfected, or (II) that multiple thermal baths be purchased to permit rapid assay of cold hardness in excised leaflets.
4. It is recommended that a search be initiated to locate sites to evaluate cold hardness under environmental conditions predominantly associated with (I) radiation frost, (II) air-drainage frost, and (III) with the influence of solar radiation on thawing.
5. It is recommended that selected freezing phenomena be identified for study at a physiological level through a suitable Contract Project coordinated with the needs of CIP.

### II. INTERMEDIATE TERM (2 - 5 years)

1. It is recommended that studies be initiated as early as possible to determine the inheritance of cold hardness.
2. It is recommended that breeding initiatives consider the integration of selected specific characteristics to be combined with cold hardness.

3. It is recommended that studies of physiology of freezing process be phased to provide a continuing program arising from Contract Project findings.

III. LONG TERM (5 - 10 years)

1. It is recommended that the possible interrelationships of resistance to cold, heat, and drought stress be evaluated.



APPENDIX I

A REVIEW OF POTATO AND HERBACEOUS PLANT HARDINESS

by

Dr. L. V. Gusta

and

Dr. M. J. Burke

## TABLE OF CONTENTS

	Page
I. Introduction .....	
II. General Considerations of Frost Tolerance .....	
III. Biochemical Considerations of Frost Tolerance .....	
A. Membranes	
B. Protein and Nucleic Acids	
C. Carbohydrates	
D. Plant and Growth Substances	
IV. Biophysical Considerations of Frost Tolerance .....	
V. Breeding for Frost Tolerance .....	
VI. Theories to Explain Frost Tolerance .....	
A. The sulfhydryl hypothesis	
B. The second supercooling point hypothesis	
C. The vital water exotherm hypothesis	
D. The mechanical stress hypothesis	
VII. Methods of Determining Frost Tolerance .....	
A. Biological methods	
1. Whole plant tests	
2. Cut leaf tests	
3. Viability tests	
a. Neutral red	
b. Conductivity method	
c. Plasmolysis technique	
d. Triphenyl tetrazolium chloride test	
B. Physical Methods	
1. Nuclear magnetic resonance	
2. Thermal analysis	
3. Electrical resistance	
VIII. Conference Objectives .....	16

## I. Introduction

The common potato (Solanum tuberosum) is considered to be one of the most important foods in the world and rivals wheat in total value. The center of origin of the potato was in the South American continent with wild tuber-bearing Solanums also occurring in Central America, Mexico and as far north as Colorado (1).

Potatoes are the staple food in the Andean region of South America. Due to the high incidence of frosts, high protein crops such as wheat, rice and corn cannot be grown successfully at the high elevations and even when frosts are no problem, potatoes yield twice as much protein per acre as wheat, rice and corn. It is an excellent dietary source of protein containing all the essential amino acids for human nutrition. Though nearly 80 percent of the tuber is water, its starch and protein content are roughly 20 and 2 percent, respectively (2).

Frosts is the major factor limiting potato production in the Andean region of South America. Depending on the intensity of the frost, either the crop may be killed or the foliage damaged, resulting in delayed maturity and reduced yields. In 1967 severe frosts in Colombia resulted in an estimated 50 million dollar loss (3).

Solanum tuberosum, the most commonly grown species in North America, possesses very little or no frost tolerance. A number of Solanum species (S. acaule; S. chomatophilum; S. commersonii; S x juzepczukii and S. multidissectum) are considered to be very frost tolerant. Attempts to incorporate this character into presently frost sensitive cultivated potatoes have lagged due to incompatibility problems and the lack of necessary screening techniques required for large segregating populations.

The objectives of this review are to discuss known data on the freezing process in plants, discuss several of the theories to explain frost hardiness and to outline some of the commonly used methods for assessing frost hardiness.

## II. General Considerations of Frost Tolerance

The resistance of plants to sub-freezing temperatures has been studied rather intensively for over a century. For recent detailed reviews on this subject, the reader should consult Levitt (4), Olien (5), Alden and Herman (6), Mazur (7), Weiser (8), Parker (9) and Meryman (10). Unfortunately in this case, most of the work has been concerned with the hardiness of woody plants which are extremely hardy. Generally, herbaceous plants cannot withstand temperatures below -20°C. The hardiness mechanism in herbaceous plants may be quite different than that in woody plants. The review of Olien

(5) deals extensively with frost tolerance of winter cereals.

In spite of the vast amount of research in this area, it is still not known how freezing kills plants and how plants adapt to freezing stress. In the case of potatoes where the level of frost tolerance is only a few degrees a reliable and quick method for screening large segregating populations has not yet been developed. This has complicated the problem of determining the inheritance of frost hardiness. Frost tolerance is an inducible genetic system controlled by low temperature. Photoperiod has been shown to be involved in many deciduous woody perennials (11-13) but not for certain woody evergreens (14) or winter cereals (15). The transition from a tender state to the hardy state is a metabolic process which requires energy. Energy is supplied through photosynthesis (5) and in the case of seedlings from energy reserves in the seed (16, 17). The problem of frost tolerance is complicated further by the fact that hardiness is not a static entity but is affected by nutrition, temperature, date and method of seeding, physiological developments, humidity, rate of growth and moisture content of tissue and soil. Although the adaptation process requires considerable time, many herbaceous species deacclimate rapidly upon exposure to warm temperatures.

There is still some dispute whether the Solanum species acclimate to frost. Several authors suggest an acclimation period of two to three weeks of cool temperatures is required to differentiate hardy from non-hardy species (18, 19); whereas other researchers have shown that potatoes have inherent frost resistance and do not further acclimate (20, 21).

In addition to low temperatures, environment may have an effect on frost tolerance in potatoes. Dry conditions (22) and low humidity (23) have been shown to markedly increase resistance. Similar results have been obtained by Metcalf et al. (24) in studying frost tolerance of winter cereals. A small increase in moisture content of the tissue can result in a very large difference in plant survival. Hudson and Idle (23) noted that a reduction in turgor of the protoplasts had a marked effect on the freezing process in S. acaule. In tissue with a high moisture content, water has nearly a single freezing point and the resulting ice crystal causes disruption of the tissue (25). Thus, if freezing occurs early in the morning, damage to the tissue would probably be greatest due to the fact that plants would have regained full turgor overnight.

Hudson (26) claimed that one of the limitations of the cut leaf test was the variability in frost resistance in leaves due to their maturity. As the leaves matured, there was a decline in frost tolerance. Mastenbrock (18) also found that the growing tip of potatoes may be killed although there was not any apparent damage to the rest of the plant. In contrast to the above findings other authors have not detected change in frost resistance with maturity (27, 28).

Other factors that may influence frost tolerance are; etiolation tends to decrease tolerance (26) and viral infection may increase tolerance by one degree (27). A rosette growth habit has been associated with winter

survival, but this does not appear to result in an increase in frost tolerance (27). This growth habit may be of benefit in the case of snow cover.

Repeated frosts have an amplifying effect on injury (25). Plants may be slightly injured when subjected to low non-lethal temperature; however, a subsequent cold treatment of lower intensity may kill the plant in its weakened condition. The ability of a plant to recover from injury is genetically controlled (5). Fully acclimated hardy winter wheat can withstand a slow freeze to  $-19^{\circ}\text{C}$ ; however, after two refreeze and thaw cycles the crown tissue will only survive  $-12^{\circ}\text{C}$  (29). Undoubtedly repeated frosts in the field will have the same effect on potatoes. Although potato seedlings were relatively unharmed by an initial frost of  $-2.8^{\circ}\text{C}$ , Ross and Rowe (30) found that two subsequent frosts of  $-1.1$  and  $-2.8^{\circ}\text{C}$  resulted in apparent injury. Hayden, Dionne and Fensom (20), measuring the electrical impedance of stem tissue from S. acaule and S. tuberosum, found a drop in electrical impedance following a slight frost. A drop in electrical impedance suggests ion leakage from cells due to membrane injury.

### III. Biochemical Considerations of Frost Tolerance

Since frost tolerance is under genetic control, certain genes are turned on during periods of low temperature to bring about frost acclimation. Elucidation of either the genes or enzymes involved may provide one way of tailoring the plant to survive frosts. Identification of key enzymes could enable the breeder to use these as markers in a breeding program. Some chemical changes which occur during cold acclimation are:

A. Membranes - During freezing, water moves from the protoplasm to the site of ice crystallization due to the free energy difference created by ice. When the energy required for ice to grow into protoplasts becomes less than the energy required to move water out, membrane injury will occur. Normally membranes are considered to be in a fluid state, but at low temperature and depending upon their chemical composition, they may undergo a phase change to a more solid state (31). This will result in a reduction of membrane permeability causing water to become trapped and freeze intercellularly (32).

In many organisms grown at low temperatures, the membrane fatty acid composition is less saturated (33-38). This increases the permeability of the membranes (35) and keeps them in a fluid state at low temperatures (39). Pomeroy, de la Roche and Miller (40), working with winter and spring wheats, reported an increase in the level of unsaturated fatty acids in the mitochondria from all varieties when grown at  $2^{\circ}\text{C}$ . It is not known if the increase in unsaturation is a low temperature growth response or just one aspect of the plants ability to adapt to low temperatures. Undoubtedly, several physiological changes are occurring during cold acclimation and if these do not occur in proper sequence or if one or more is lacking, then

the full hardiness potential is not expressed.

Olien (5) has shown that ice penetrates non-hardy protoplast easier than hardy protoplasts. Membranes from hardy tissue are more elastic than membranes from non-hardy plants and therefore are able to withstand the stresses induced by the growing ice crystal.

Phospholipid changes occur during cold acclimation of black locust (41) and alfalfa (36). However, de la Roche, Andrews and Kates (17), using various techniques for phospholipid extraction, could not detect any difference in phospholipid composition (i. e. phospholipid class) in wheat seedlings grown at 24 and 2°C.

B. Protein and Nucleic Acids - The role of proteins, either in the catalysis of metabolic reactions or in maintaining the structural organization of membranes, suggests an intimate involvement in the development of frost tolerance. Qualitative (42-44) and quantitative (45-47) changes occur during cold acclimation of many plants. However, other researchers reported no detectable changes in protein (4, 48).

Many of the current hypotheses proposed to explain cold resistance and injury in one way or another are centered around the role of proteins and their involvement in the cell's structural integrity. Certain enzymes are known to be cold labile. For example, glycogen phosphorylase (49), lipoxidase (50), D-amino acid oxidase (51), phosphatase and peroxidases (52) undergo reversible partial inactivation in vitro at low temperatures. Irreversible cold inactivation has been shown for frog carbamyl phosphate synthetase (53) and beef mitochondria ATPase (54). Certain lipoproteins cannot be frozen without denaturation and loss of characteristic solubility properties (55).

Certain proteins have a protective effect during freezing. Williams (56) reported that during cold acclimation of Cornus florida, a glycoprotein is synthesized which is capable of binding large amounts of water. Herber (57) has isolated two proteins from spinach leaves which were more effective than sugars in protecting chloroplasts. DeVries (58) was able to show that certain glycoproteins protected arctic fish against freezing injury.

An increase in RNase occurs in leaf tissue subjected to a stress. Cold (59), insect infestation (60), osmotic shock (61) and excision (62) resulted in dramatic increases in RNase activity in a variety of plants. A rapid loss of all RNA species occurs in boxwood leaves subjected to a lethal frost (46). Recent research has indicated cryoinjury in animal cells may result from rapid enzymatic degradation of cell constituents following disruption of intracellular compartmentalization (63). Although RNase may not be directly involved in cold injury, it certainly may have a role in recovery.

Quantitative and qualitative changes in ribonucleic acids occur during cold acclimation of woody plants (41, 46, 47). With the development of the full hardiness potential, there is a marked reduction in rate of metabolism of radioactive nucleic acid precursors into RNA in barley (64), wheat (65) and boxwood (66) leaves. This is in support of the evidence

that growth cessation is required for complete acclimation.

Li and Weiser (67), working with frost sensitive *S. tuberosum*, reported an increase in all RNA species when the plants were grown under short days and low temperatures. Short days and low temperatures also stimulated an increase of  $^{32}\text{P}$  incorporation into all RNA species (68).

Purines and pyrimidines increase cold hardiness, proteins and nucleic acids in alfalfa crowns (69). However, certain purines enhance low temperature breakdown in apples (70).

C. Carbohydrates. - In many plants, sugars increase in the fall as plants acclimate to cold and decrease in the spring during deacclimation (71). This has led many researchers to suggest that sugars have a causal role in cold hardiness (72-74). Also, if non-hardy plants are incubated in sugar solutions, there is generally an increase in frost tolerance (75-77). Sugars have also been shown to protect isolated spinach chloroplasts (74). Steponkus (72) suggested that during hardening an alteration in protein structure increases this affinity for sugars. The bound sugars are then able to protect against protein denaturation during freeze-dehydration.

However, an increase in sugars does not always parallel the increase in frost tolerance (4). Fuchigami, Weiser and Richardson (78) were unable to show an increase in frost tolerance of red-osier dogwood with various levels of sugars fed continuously. Also in the case of potato plants, low temperatures increased the concentration of sugars but there was no apparent effect on frost resistance (78).

Xylans, high molecular weight carbohydrates, have a direct effect on ice crystal growth and on the type of ice structure formed (79). Olien (5) has shown that these xylans compete with water for sites on the growing ice crystal. Sakai (80) has identified several polyhydric alcohols which act as antifreeze agents.

D. Plant Growth Substances. - During cold acclimation there is a cessation of growth or slowing down of growth. Many herbaceous plants adapt a rosette growth habit or appear stunted. These observations suggest that during cold acclimation there is a reduction of growth promoting hormones. Cold tolerance of winter cereals has been reduced by exogenous application of gibberellic acid ( $\text{GA}_3$ ) (81). Applications of 2-chloroethyl trimethyl ammonium chloride (CCC) to winter wheat induces small increases in cold hardiness (82). Exogenous applications of CCC reduce the gibberellin content of many plants (83-85) by inhibiting certain steps in gibberellin synthesis. This led Roberts (82) to suggest that during cold acclimation of winter wheat there is a reduction of endogenous gibberellins.

Exogenous applications of IAA to winter wheat increased the content of natural auxins, decreased growth inhibitors and stimulated plant growth (86). This resulted in a loss of frost resistance of winter wheat plants grown at  $5^\circ\text{C}$  but not at  $0^\circ\text{C}$ . Cold treatment of winter wheat seedlings resulted in a 10-fold increase in indoleacetic acid oxidase (87). An increase in IAA oxidase would tend to keep the endogenous auxin at a low level and thereby prevent the stimulation of growth and the concomitant loss of cold tolerance.

Abscissic acids (ABA), a natural growth inhibitor, increased the hardiness level of *Acer negundo* (88). ABA has been implicated in the regulation of water balance in plants by inducing stomatal closure and increasing water permeability (89-92). The content of endogenous ABA was found to be very responsive to water stress (91) and mineral starvation (93). Cytokinins on the other hand appear to act as a check on ABA. Evidence suggests that cytokinins tend to open stomata and reduce membrane permeability to water. Both membrane permeability and water content have been implicated in frost tolerance of potatoes.

#### IV. Biophysical Considerations of Frost Tolerance

Upon freezing of tissue, the cell water will supercool if there are no ice nucleation sites. MacKenzie and Rasmussen (94), have shown that cells are not ice nucleators; therefore, ice usually forms in the extracellular ice crystal grows, water moves from the cell due to the free energy differences created by the ice. As more water is lost the cells start to contract and become dehydrated (4). The shape of the ice crystal depends on the extent of supercooling, and the rate of temperature decline. With slow cooling and no supercooling, amorphous ice crystals are formed which are considered noninjurious to the cell (4, 5). No two ice crystals have the same shape and different shapes have different effects on the distribution of the plant's water and also on the ease with which they penetrate the protoplasm (95). This may help explain the wide range of killing temperature reported for potatoes (26).

Crystallization does not proceed into the protoplasts of hardy cells because the plasmalemma acts as a barrier to ice crystal growth (5, 71). In the case of tender plants, extracellular ice grows through the protoplast, resulting in death of the cells (96). During frost acclimation the plasmalemma undergoes a transition and becomes a barrier to ice growth. However, this is not the only reason why plants become frost tolerant. Olien (25) identified five basic patterns of water redistribution in barley plants during freezing. The water redistribution patterns were dependent on the rate of freezing, the resistance of the protoplasm to ice penetration, the moisture content of the cell, the pattern of initial ice growth in the tissue and various other factors which contribute to the hardiness of the cell. Depending upon the above conditions, Olien was able to demonstrate that freezing occurred either as an equilibrium or non-equilibrium process. Non equilibrium freezing was asso-



ciated with tender or semi-tender plants. Sukumaran and Weiser (97) were able to show that freezing occurred as a non-equilibrium process in S. tuberosum and as a semi-equilibrium process in S. acaule.

From the freezing patterns observed in this study, it would appear that the injury occurred during freezing and not in the thawing stage. However, Hudson (26) found that a rapid thaw resulted in a greater variation in survival than a slow thaw.

Hudson and Idle (23) followed the formation of ice in the petioles of S. acaule and S. tuberosum by light microscopy and by differential thermal analysis. Their results showed that S. acaule freezes in two distinct stages, whereas the freezing process in S. tuberosum was a more continuous process. In S. acaule ice first formed in the vascular regions and gradually formed in the subhypodermal tissue. In S. tuberosum two exotherms were not evident because ice was laid down at scattered loci throughout the tissue. Sukumaran and Weiser (97) were unable to detect two exotherms during the freezing of S. acaule leaves. Perhaps this was due to the different tissues tested.

Olien (5) considers that water associated with hydrophilic plant components is critical for the survival of plant tissue during freezing. Many hydrophilic plant components such as carbohydrates, proteins and nucleic acids tend to structure water about themselves (98). The range of this induced structure is uncertain, but Kavanau (98) suggests it will depend on the components involved. During the initial stages of freezing, water which is least affected by hydrophilic plant components has nearly a single freezing point. If the temperature is held constant, an equilibrium is established between the ice crystal and the various plant components for the remaining water. With a steadily decreasing temperature, the equilibrium is shifted in favor of the growing ice crystal. Thus the more hydrophilic components a cell has, the greater its chances for survival.

Various other plant components are known to have an effect on water. Certain small inorganic ions tend to cause the breakdown of tetrahedral water by reorientation and immobilization of water in their vicinity (99). If the concentration of these inorganic ions is high enough, a salting out of solutes will result (100). Also certain small hydrophobically hydrated ions promote water structure (99, 101). Many macromolecules, e.g., proteins and membranes, are able to structure water into so-called "iceberg" (102). This water is considered essential in maintaining the structural integrity of native macromolecules. Low temperature instability of proteins and membranes has been associated with the weakening of hydrophobic bonds at low temperatures (103, 104). Frozen membranes readily fracture in their inner hydrophobic regions due to a weakening of the hydrophobic bonds (105).

In addition to certain plant components affecting water structure, certain plant gums and xylans have a direct effect on freezing survival (106, 107). Olien (5) has termed these gums as ice kinetic inhibitors which affect frost survival by controlling the site of ice formation and the type of ice structure that develops.

An increase in membrane permeability during frost acclimation has been suggested as one means of avoiding injury (71). Hudson and Idle (23) propose that the high permeability of S. acaule cells allows solutes to readily diffuse to the extracellular ice and to initiate a thaw. This thaw accounts for the two stages of ice freezing. Sukumaran and Weiser (97) measured the water permeability of hardy S. acaule and tender S. tuberosum potatoes and found no substantial difference. Hudson and Idle (23) postulated that ice formation itself raises the permeability of the cells. Maheshwari and Sussman (108), studying cold-induced dormancy in urediospores, believe that temperature causes physical changes in the lipoprotein of cytoplasmic membranes and thus alters their permeability. Ring (109) also found that low temperatures resulted in the widening of pores in membranes which increased their permeability. Winter wheat plants hardy to  $-18^{\circ}\text{C}$ , when subjected to a frost of  $-5^{\circ}\text{C}$ , lose ions at a greater rate than unfrozen controls (110). An increase in ion leakage occurred when several frost resistant potato cultivars were subjected to low non-lethal temperature (111). It is difficult to assess if this was due to increased membrane permeability or to death of a few cells which did not affect survival.

In summary, the water content, the effect of plant components on water, patterns of initial ice growth in the tissue; the redistribution of water during freezing and the permeability of membranes are major characteristics affecting stress and are determined by genetic and environmental variables.

## V. Breeding for Frost Resistance

Since a large number of the wild Solanum species have frost resistance, there is considerable genetic potential for incorporating this character into cultivated species (See Mastenbrock (18) and Richardson and Weiser (3) for reviews of frost resistant species). The inheritance of frost resistance is not well understood, perhaps due mainly to the lack of suitable means of screening segregating populations for frost resistance and tetrasomic inheritance. Mastenbrock (18) suggests that the genes for frost resistance are dominant and one or a few genes are in-

volved which are quantitative or cumulative in effect. Ross and Rowe (30) working with frost-resistant diploids, reported that the  $F_1$  progenies and  $F_1 \times F_1$  progenies segregated to produce plants with a greater frost tolerance than the parental species. This would suggest that inheritance for frost tolerance is quantitative and transgressive segregation may be involved. In the same study these authors found that when the hybrids from the wild species were crossed to haploid S. tuberosum, the frequency of frost resistance in their progeny decreased approximately 50 percent. Bloomquist and Lauer (112) reported a decline in frost resistance of S. acaule x S. tuberosum hybrids and backcross derivatives as the proportion of S. acaule genes decreased. This would suggest that genes with small additive effects are involved.

S. acaule is one of the most frost resistant Solanum species (18, 26, 113, 114). Although S. acaule is a tetraploid, attempts to cross it with S. tuberosum have met with limited success (18, 115). Mastenbrock (18) partially overcame this difficulty by using a cross of two types of S. acaule. The resulting hybrids could then be crossed more readily with S. tuberosum.

Many of the frost resistant wild Solanum species are either poor yielders or do not produce tubers. This necessitates a backcross program for tuber appearance and yield. Another problem is wild species are more photoperiodic sensitive than S. tuberosum. Long days are required for flower initiation and short days for tuber production. Rudolf (115) found that after one backcross to S. tuberosum, the hybrids shifted to the behavior of S. tuberosum.

Over 60 percent of the tuber bearing Solanum species are diploid, which represent a large storehouse of relatively untapped germplasm. Attempts to form tetraploids from crosses with S. tuberosum have met with limited success. The introduction of haploids ( $2n=24$ ) of S. tuberosum L. ( $2n=48$ ) by Hougas and Pélouquin (116) provided an effective means for gene transfer from tuber bearing diploids to S. tuberosum. However, the pollen fertility of many of the haploids is relatively low and thus the haploids are used as the pistillate parent. If frost tolerance of potatoes is maternally inherited as suggested by Hudson (26) this method may have limited application. The haploid, US-W4, as shown by Van Suchtelen (117), is reasonably fertile. This clone was used to pollinate other haploids and most of the plants obtained formed flowers with fertile pollen.

Van Suchtelen and Verdenius (118) crossed haploids of S. tuberosum with frost resistant diploid S. ajanhuiri and obtained hybrids which survived field frosts of  $-3^{\circ}\text{C}$ .

Colchicine is widely used to induce chromosome doubling in plants. However, treatment of potatoes with colchicine frequently results in a low and variable frequency of recoverable doubled clones (119). In addition to its toxicity, mutant plants arise from colchicine treatment (120, 121).

Ostergren (122) reported nitrous oxide was effective in producing polyploid Pisum sativum and Crepis capillaris if the zygotes were treated at the time of zygotic division. Nitrous oxide, applied under pressure, rapidly penetrates cells and when the pressure is released, the gas is rapidly released from the cells. This permits a finer control of treatment in comparison to colchicine. Dvorak, Harvey and Coulman (123) found nitrous oxide to be very effective as a polyploidizing agent in barley and wheat. To the authors knowledge, nitrous oxide has not been used to produce polyploids in potatoes.

## VI. Theories to Explain Frost Tolerance

Numerous theories have been proposed to account for cold hardiness in plants. A common denominator to many is the problem of water removal from the cell during freezing. Ice crystals, growing extra cellularly, cause dehydration and cellular contraction as freezing proceeds. This dehydration and contraction can lead to mechanical stress on the cell as well as concentration of the cell constituents such as proteins, salts, sugars, organic acids, etc. Described below are four theories of cold hardiness which are often cited in the literature. These theories may or may not have relevance to potato cold hardiness.

A. The sulfhydryl hypothesis - Levitt (4) proposed the sulfhydryl hypothesis. This hypothesis assumes that, because protein molecules come very close together in freeze dehydrated cells, oxidation of the sulfhydryl groups occurs on adjacent protein molecules. This oxidation results in disulfide bond formation between different protein molecules. The reaction is irreversible and thus denatures the proteins of the cell. Freezing tolerance is related to events occurring during cold acclimation which prevent disulfide bond formation.

B. The second supercooling point hypothesis - Tumanov and Krasavtsev (32) have suggested that at certain temperatures during freezing, water is restricted by the plasmalemma from moving to the extra-cellular ice. This results in supercooling of water in the protoplast until nucleation occurs which results in intracellular freezing. They proposed this hypothesis for plants which have low temperature exotherms.

C. The vital water exotherm hypothesis - Weiser (8) proposed the vital water hypothesis which in some ways is similar to the second supercooling point hypothesis. Weiser suggests that a certain amount of water is required by the protoplast to maintain structural integrity. During

freezing a point is reached where all the readily available water has been removed by extracellular freezing. Upon freezing, vital water is pulled away from protoplasmic constituents to the extracellular ice. This results in a chain reaction of denaturation, additional vital water loss and death.

D. The mechanical stress hypothesis - Iljin (124) proposed the mechanical stress hypothesis. He noted the collapse of cell walls which accompanied the formation and growth of ice in the extracellular spaces. On thawing of these tissues he observed that the cell wall snapped back to its original position which often tore the plasmalemma. Iljin concluded that the injury occurred only during thawing. There are certainly many exceptions to Iljin's hypothesis; however, it dramatizes the importance of controlled and slow thawing of frozen tissues.

## VII. Methods of Determining Frost Tolerance

A. Biological Methods - The classification of plant populations exposed to natural environments is perhaps the oldest and ultimate test for evaluating cold hardiness. However, seedling populations are exposed to the irreproducible and unreliable selection pressure of natural frost which makes results difficult to interpret. Due to microclimatological factors resulting from air drainage, difference in foliage cover and height of the plants and variation in terrain, it is difficult to obtain a uniform frost. In the case of severe frost, the entire breeding population may be lost.

Controlled freeze tests permit greater control over experimental conditions and the experiments can be replicated over time. Several parameters must be considered in using a controlled freeze test to evaluate survival. Many types, combinations and sequences of stress occur as a result of normal environmental variation. A plant may have the ability to survive one type of stress and yet not have the ability to survive others. These stresses must be recognized and duplicated in a controlled freeze test. Listed below are several artificial methods used to assess and/or predict frost tolerance. The ideal method should be quick, simple, repeatable and nondestructive. Unfortunately, to date there is no test available which meets all of these criteria.

1. Whole plant tests - This method is perhaps the simplest in preparation. Whole plants are frozen in controlled freezer chambers at a predetermined rate to a series of test-temperatures. The time interval at which the plants are maintained at a certain test temperature varies with the researcher (18, 113). The plants to be frozen are either in flats

(18) or pots (18, 113) or removed from soil (125). Upon completion of the test, the plants are thawed and then rated for injury by either regrowth (125), visual damage (113) or leakage of cell constituents (111).

Although this method appears rather simple and straightforward, there are several serious drawbacks. The researcher must have access to the plants in the chamber to initiate freezing. There must be uniform temperature distribution throughout the chamber. A variation of  $\pm 0.5^{\circ}\text{C}$  could give misleading results. Plants in pots with a rosette growth habit could be protected by the large heat capacity of soil. In the case when the plants are lifted and free of soil, injury may occur in the root initials. Thus a regrowth test would not be possible. In many plants such as winter cereals the root initials are killed at a warmer temperature than the foliage (126). Large variation in survival may occur with the use of flats. Generally, those plants located at the edge of the flat show greatest injury.

Perhaps the biggest drawback in using whole plants is that the material being tested is sacrificed. Thus, a large population of plants is required which may not be possible with certain segregating populations. To ensure reproductibility of results, the test must be replicated within each test run and lines of known resistance must be included to serve as controls. The arrangement of plants must not give rise to altered air circulation and temperature variations.

In order to screen large populations, a rather large and costly freezer chamber is required. However, the larger a chamber is, the more difficult it is to maintain uniform temperature and humidity. A reliable screening test for whole plants must have very precise control of temperature change must be constant and linear and at the rate desired. There should also be some way to initiate freezing of the tissue just below  $0^{\circ}\text{C}$ .

2. Cut leaf test - Although the cut leaf test is rather lengthy and requires good temperature control, it is relatively simple and non-destructive to the whole plant. According to Hudson (26), the cut leaf tests were unreliable due to irregularities in nucleation which resulted in supercooling. Hudson (26) could not distinguish resistant and non-resistant lines unless supercooling was prevented. Asahina (127) reported that sprouts from potato tubers remained supercooled for 18 hr. at  $-5.5^{\circ}\text{C}$  and for four hours at  $-7.5^{\circ}\text{C}$ . If the foliage was inoculated with ice crystals at  $-6^{\circ}\text{C}$ , freezing occurred intracellularly resulting in death of the cells. When the tissue sections were inoculated at  $-1.8^{\circ}\text{C}$ , freezing occurred extracellularly and the tissue resisted freezing. Sukumaran and Weiser (111) nucleated their samples at  $-2^{\circ}\text{C}$  on the upper surface of the leaves, whereas Hudson (26) immersed the cut end of petioles in crushed ice. According to Olien (5), nucleation usually occurs on the upper surface of leaves in the field. It is not known if the site of nucleation has an effect on the degree or type of frost injury. However, immersion of the cut end of petioles in water overnight could raise the cellular water content and have an effect on frost tolerance.

Hudson (26) concluded the main limitation of the cut leaf test was the differences in hardness exhibited by leaves at different stages of development. To overcome this, Sukumaran and Weiser (111) selected composite samples representing leaves of different ages. Sukumaran and Weiser (111) obtained good agreement between the cut leaf test and whole plant tests. Injury was estimated quantitatively by electrolyte leakage. Various species (*S. acaule*, *S. chomatophilum* (266387 and 243340) could tolerate freezing to  $-5.5^{\circ}\text{C}$ , which was in general agreement with previous reports (3). Blomquist and Lauer (112) evaluated frost resistance using the cut leaf test and found the results were consistent with observations made during natural field frosts. Although the cut leaf is somewhat lengthy, it still appears the most reliable method of assessing frost tolerance for potatoes.

Some difficulties of the cut leaf test may be avoided by use of a temperature bar as described by Timbers and Hocking (128). A temperature bar is a flat rectangular surface (10 ft. by 2 ft.) whose temperature can be regulated as a linear gradient from one end to the other. For potato studies the temperature of such a bar would run linearly from  $0^{\circ}\text{C}$  at one end to  $-10^{\circ}\text{C}$  at the other. Samples from a single test plant are distributed in uniform intervals from one end of the bar to the other. The closer samples are placed along the length of the bar the finer the hardness determination. The bar is initially at  $0^{\circ}\text{C}$ , the samples are placed on the bar then covered by a layer of damp cheese cloth and by an insulating styrofoam cover. The cheese cloth will freeze at just below  $0^{\circ}\text{C}$ , initiating ice nucleation and preventing supercooling of the leaf tissue. The temperature gradient is then generated over a period of several hours to keep cooling rates slow. Warming rates can be controlled in a similar fashion. At the end of the experiment the samples can be tested by visual observation for viability. Depending on the size of the bar, hundreds of samples can be tested in a period of several hours.

3. Viability tests - Visual observations of freezing injury are the quickest and simplest, but may suffer from bias. Injury to tender plants is reflected by a water-soaked appearance of the tissue upon thawing (26). However, some plants may have a water-soaked appearance initially but eventually recover. Tissue browning generally requires an incubation period of a week, but is very reliable for a range of plants (129). Regrowth is perhaps the ultimate test, but is rather lengthy (3 to 4 weeks) and considerable space is required. The tests listed below are mainly objective and relatively quantitative. Not all of the tests are universal and they

should be compared to regrowth tests to ensure that the test is valid.

a. Neutral red. At a neutral pH this dye is not ionized and readily enters both live and dead cells. In living cells, neutral red is ionized and retained in the cell whereas in dead cells the dye tends to readily leak out (71).

b. Conductivity method. This method is based on the amount of electrolyte which diffuses out of the cells following cold exposure (130). Electrolytes diffuse more freely from injured cells. The lethal temperature is generally regarded at the temperature when 50 percent leakage occurs. Sukumaran and Weiser (111) were able to distinguish the killing temperature of S. tuberosum and S. acaule using this method. A variation of the electrolyte conductivity method is the leakage of amino acids from cells following frost exposure (45).

c. Plasmolysis technique. Healthy cells readily plasmolyze in a hypertonic solution such as calcium chloride. When cells have been injured by freezing, the selective permeability of the membrane is lost and the cells do not plasmolyze. Microscopic examination of this tissue reveals the extent of injury and also which class of cells has been injured (5).

d. Triphenyl tetrazolium chloride test (TTC). This method is based on the reducing capacity of cells which is eventually lost after injury (131). If cells are healthy, the oxidized form of TTC, which is colorless, is reduced to the red form quickly by the cell. After an incubation period the reduced dye is extracted with 95 percent ethanol and quantified photometrically.

#### B. Physical Methods -

1. Nuclear magnetic resonance - Nuclear magnetic resonance (NMR) provides a very simple, quick and nondestructive way of studying tissue water. It can be used to study the status of unfrozen tissue water in terms of oriented or structured water (bound water) and it can be used to measure the amount of unfrozen water present at sub-zero temperatures. There have been several studies which have implied that bound water is involved with cold hardiness (132, 133); however, Burke et al. (134) using NMR, could not detect any evidence that bound water played a large role in cold hardiness of Cornus stolonifera. The quantity of unfrozen water between -15° and -30° C (on a dry weight basis) was not dependent on the cold hardiness of the tissue. The amount of ice formed in tender tissue is several fold higher than in hardy tissue since there is much less total water in hardy stems. A similar conclusion based on calorimetric measurements was reached for winter wheat (135). Gusta and Russell (136), using continuous wave NMR measurements detected a broadening of the NMR absorption band for water during cold acclimation of winter wheat.



During deacclimation there was a reversal in line width. NMR will certainly be of use in understanding the dynamic and freezing properties of tissue water. There are two methods of NMR; continuous wave NMR and pulsed NMR. In both of these methods, a sample is placed in a test tube (0.3 to 1.0 cm in diameter) and inserted into the instrument. Besides the fact that the tissue must be excised, the method is non-destructive to the tissue.

Continuous wave NMR is radiowave absorption spectroscopy requiring a magnetic field and depending on the strength of magnetic field used, resonance of protons occurs between 10 MHz to 330 MHz (commercially available spectrometers). There are two types of continuous wave spectrometers. In the most common type, the radiowave light source is held at a constant frequency and the magnetic field strength is varied. At certain field strengths, resonance occurs and at resonance an absorbance line is obtained. Alternatively in the second type of spectrometer, the magnetic field intensity is held constant and the radiowave frequency of the light source is varied slowly through the resonance range.

The NMR spectrum is a plot of absorbance on the ordinate vs frequency and/or magnetic field intensity on the abscissa (Fig. 1). The integrated absorption intensity (area under the absorption curves) is proportional to the liquid water content of the sample (Equation 1). In this equation,  $L$  equals the total water present (percentage of fresh weight),  $L_t$  equals the liquid water present at temperature  $T$  (total water - ice);  $A_{10}$  equals the integrated absorption intensity at 10°C,  $A_t$  equals the integrated absorption intensity at temperature  $T$ , and  $T$  equals temperature in °K.

$$L = L_t \frac{A_t}{A_{10}} \frac{283}{T}$$

In Fig. 1 examples of an NMR absorption spectrum (a) and the freezing curve (b) obtained from such spectra are given. In Fig. 2, the absorption line becomes broader as the temperature is reduced, thus making integration of the curve progressively more difficult. The line width is proportional to the rotational correlation for water (an expression of the ease of rotation of water molecules) and therefore potentially provides information on the amount of "bound" water in the plant tissue; however, attempting to attribute line width solely to "bound" water content of the tissue is subject to considerable error. Not the least difficulty arises from defining the term "bound" water. Some of the problems of line width interpretations can be avoided by the use of pulsed NMR.

In contrast to continuous wave NMR, both the radiowave frequency of the light source and the magnetic field intensity are adjusted in pulsed NMR

spectroscopy. In the experiment the sample which is in the magnetic field of the spectrometer is exposed to a brief pulse (usually less than 10  $\mu$ sec) of radio frequency light. The sample absorbs the light, goes into an excited state which is metastable and decays with time. Because the decay time is short, the signals are monitored on an oscilloscope. Two kinds of pulses are commonly used, 90° and 180° pulses. In the magnetic field of a pulse spectrometer the protons are predominantly aligned with the magnetic field of the spectrometer. After a 90° pulse, protons are oriented perpendicular to the magnetic field of the spectrometer. After a 180° pulse, the protons are oriented antiparallel (against) the magnetic field. The electronics of the spectrometer are such that only protons which are oriented perpendicular can be observed, therefore, following a 90° pulse a signal is observed which decays with time. The decay process after a 90° pulse is called free induction decay. After a 180° pulse no signal is observed although the direction of the protons orientation is changes.

Two relaxation times are commonly measured on the pulsed spectrometer. They are spin-spin ( $T_2$ ) and spin-lattice ( $T_1$ ) relaxation times. These relaxation times provide a measure of the dynamic properties of the tissue water such as the fraction of water bound, its viscosity and self-diffusion coefficient. Combinations of 90° and 180° pulses are needed for these measurements. Good reviews of these relaxation processes and their interpretation can be found elsewhere (137). The half life of the free induction decay of ice and most nonaqueous components of biological tissues is very short, usually less than 10  $\mu$ sec. Conversely, liquid water has a longer half life (milliseconds to seconds). Therefore, if one monitors the free induction decay 15  $\mu$ sec after the 90° pulse, the only signal remaining results from the liquid water. That signal is analogous to the integrated absorption intensity,  $A_t$ , in equation 1. This output signal,  $A_t$ , can be fed into a recorder and the liquid water content can then be recorded directly as the sample is cooled or warmed.

2. Thermal Analysis - The freezing of water is an exothermic reaction. This property of water has been used by several researchers to determine at what temperatures substantial amounts of water freeze in tissues and if any of the observed exotherms are related to injury (23, 138-142). Heat which is released by the crystallization of water can be measured by calorimetry, fine thermocouples, etc.

Exotherm studies have several advantages in that they are relatively quick, only small samples are required and the results are available in 2 to 6 hours. Unfortunately, only in a few instances has it been possible to demonstrate that exothermic changes are associated with freezing injury to hardy plants. Tumanov and Krasavtsev (138) using calorimetric and microscopic studies during freezing of stems, demonstrated a calorimetric lag in the freezing processes at temperatures slightly above the killing point and a discrete release of heat and a loss of fluorescence in cells at

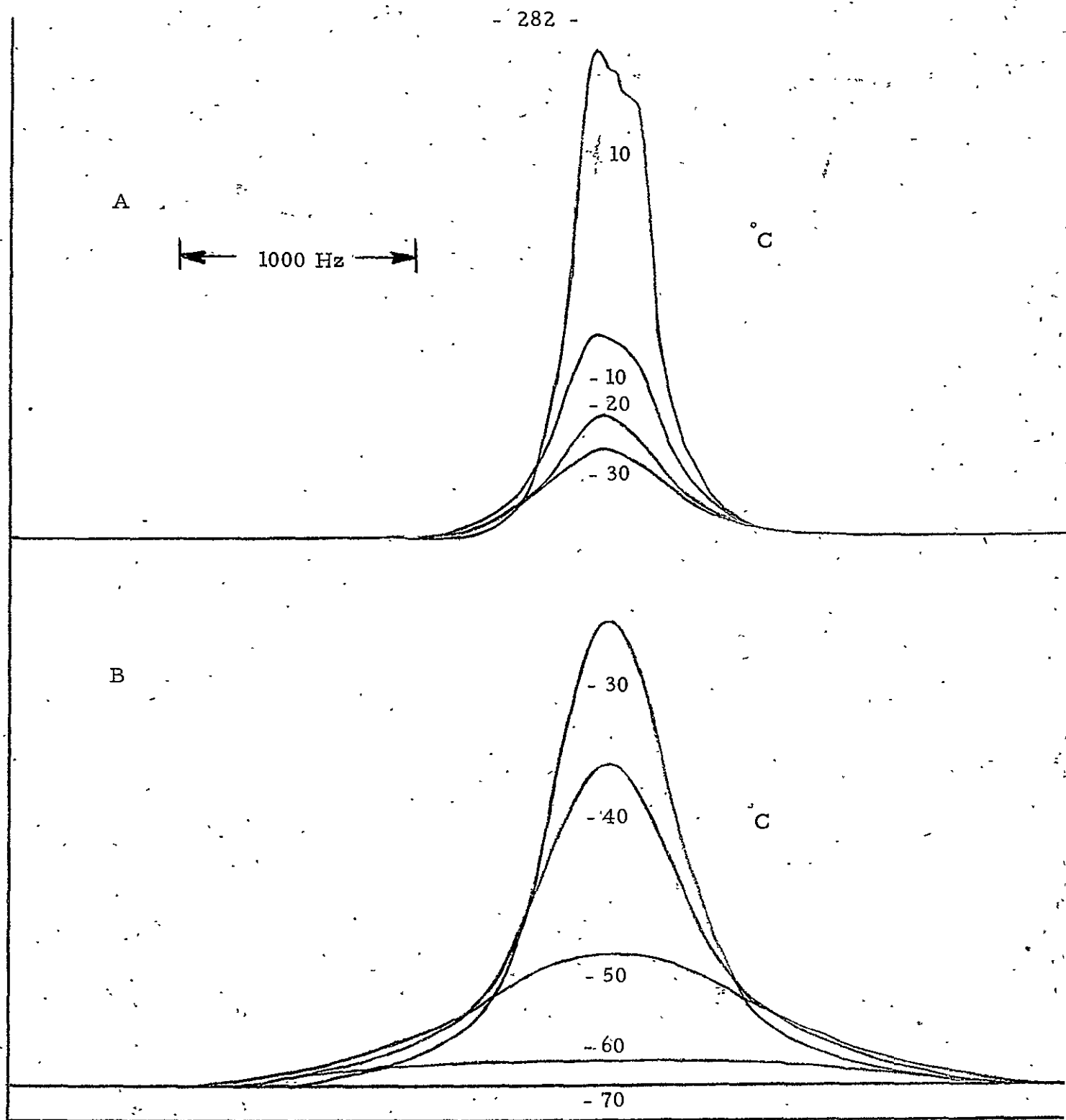


Fig. 1. 100 MHz NMR spectra of an acclimated dogwood stem. a) Spectra between +10°C and -30°C. b) Spectra between -30°C and -70°C. In b the radio frequency field strength was increased by approximately 13-fold which increased the sensitivity. The cylindrical sample was stationary and perpendicular to the external magnetic field.

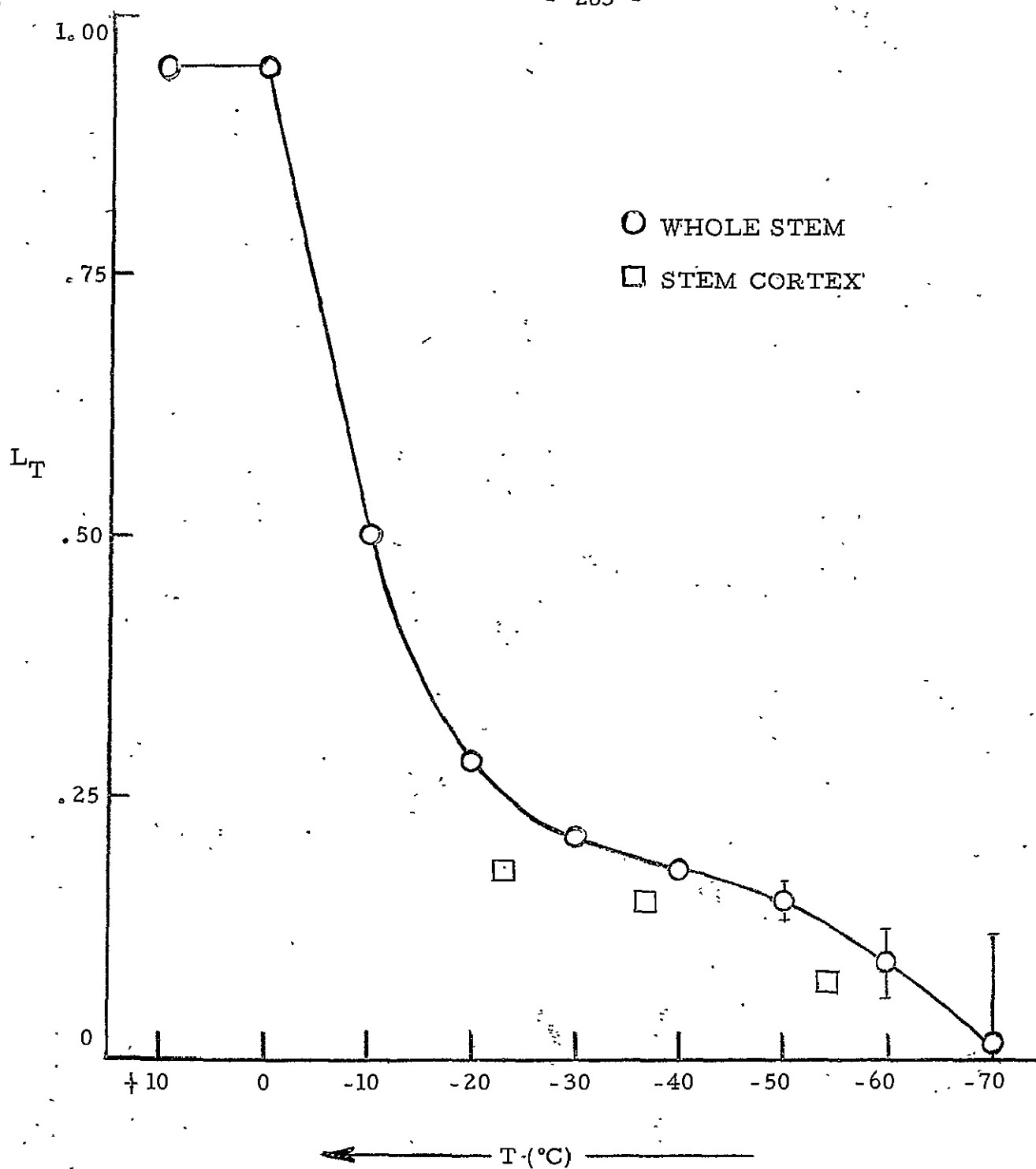


Figure 2. Freezing curve of hardy dogwood stem and hardy dogwood bark. The data were obtained by integrating 40000 Hz of the continuous wave NMR spectra at 100 MHz.  $L_T$  is defined in Equation 1 and is expressed in grams liquid water per gram dry sample. The spectra used are those in Figure 1. The full band width never exceeded 8000 Hz.

the moment of injury. Graham (141) working with hardy deciduous azalea buds reported a distinct exotherm which was associated with injury. Quamme, Weiser and Stushnoff (142) using differential thermal analysis found an exotherm at low temperature which was associated with injury to the xylem and pith. Hudson and Idle (23) were able to detect two distinct exotherms during slow freezing of S. acaule petioles. In S. tuberosum these two exotherms were less well defined. By following the process of freezing by light microscopy in S. acaule and S. tuberosum, Hudson and Idle (23) were able to relate the exotherms to the patterns of freezing in the tissue. S. acaule first froze in the vascular tissues and then in the adjacent extracellular spaces whereas in S. tuberosum ice forms at scattered sites throughout the tissue. Sukumaran and Weiser (97) working with the same two species could only detect one exotherm in their thermal differential freezing profiles. In this study the thermojunctions were affixed to the leaf surface whereas Hudson and Idle (23) had their thermojunction inserted in the petiole.

Three methods are used for thermal analysis, 1) thermal analysis as such, 2) differential thermal analysis and 3) differential scanning calorimetry. These methods are used to determine the freezing and thawing points of tissue water. Differential scanning calorimetry is also used to estimate the fraction of water frozen.

The equipment required for thermal analysis includes a thermocouple for sample temperature measurement, a temperature recorder and a device to cool the samples at a controllable rate. This method is used to show the freezing point of leaf tissue and also to make a qualitative estimate of rate of tissue water freezing.

Differential thermal analysis differs from thermal analysis in that both a reference sample of known thermal characteristics and the sample are cooled simultaneously and the temperature difference between the two is recorded. This is usually performed using two thermocouples in series, one in the reference and one in the sample. Data from differential thermal analysis is generally plotted as the temperature difference between sample and reference on the ordinate vs sample temperature, reference temperature or time on the abscissa. This technique is applied in the same fashion as thermal analysis; however, the differential method is more sensitive. Both methods provide only a qualitative estimate of the quantity of frozen water at various temperatures. The chief advantage of these methods is the simplicity of the equipment necessary for the experiment.

Differential scanning calorimetry has been applied to cold hardiness research, and has employed two types of calorimeters, the Calvet calorimeter used by Krasavtsev and Olien and the more conventional scanning calorimeter such as the Perkin-Elmer, Dupont, Stone, etc. These instruments perform the same tasks as thermal or differential thermal analysis instru-

ments do with the added feature of determining the amount of water frozen or thawed between any two temperatures. Unlike the other thermal analysis methods which measure temperature or temperature difference, the differential scanning calorimeter measures the difference in the heat evolved or absorbed during cooling, or warming between an unknown sample and known reference. The reference varies from air to metals depending on the sample requirements. The amount of water frozen can be determined from the heat evolved during cooling using the heat of fusion of water and the heat capacities of ice and liquid water. The main weakness in calorimetric measurements is in the choosing of heats of fusion and heat capacities for tissue water, particularly for the liquid water remaining at low temperature in a partially frozen tissue. For potato foliage where interest is focused in the 0° to -10°C temperature range, these above difficulties are probably minor. Another factor making any error from the choosing of these terms minor in potato studies is that any study of potato will be a comparative study between the hardy and tender species. Thus, the magnitude of errors introduced by incorrect heat capacities or heats of fusion will tend to be attenuated so long as comparison measurements are used.

3. Electrical resistance - Electrical measurements have been shown to be a useful procedure for the evaluation of frost hardiness in certain plant species (20, 143-145). In 1931, Luyet found a decrease in electrical impedance of plants after the tissues had been injured by cold, heat or lipid solvents (146). Luyet attributed the decrease in electrical impedance observed at low frequencies to the degree of destruction. Greenham and Daday (147) working with white clover and alfalfa, attributed the drop in electrical impedance following cold injury to the destruction of the plasma-lemma.

Electrical measurements on woody tissue to assess winter hardiness have had varying degrees of success. Weaver et al. (148), working with peach scions, could not separate different hardiness groups. Also the method was restricted to bearing trees. Craig, Gass and Fensom (149) were unable to differentiate between winter hardy and tender cultivars of raspberry due to extensive physiological changes occurring during development. Hayden, Dionne and Fensom (20) reported that electrical impedance measurements of the stems or petioles of Solanum clones were a reliable method of assessing relative frost hardiness. However, in order to make valid comparisons between clones, the plants had to be grown and tested under carefully controlled conditions and at least 10 plants were required for averaging.

In the studies discussed above, most of the electrical measurements were done at a single low frequency and without temperature control.

Measurements at low frequencies are influenced by membranes changes (145) and changes in stem diameter (148), electrolyte concentration (146), cell size (147) and changes in temperature (150). To minimize these variables Evert and Weiser (145) used a ratio of a high and a low frequency impedance for predicting the cold hardness of stem sections of red-osier dogwood.

Electrical impedance measurements offer practical advantages over the previous techniques because they are determined with ease without excising the tissue. Impedance with alternating current is analogous to resistance with direct current. In contrast to previous methods which provide information on the freezing water, electrical impedance measurements provide information on the integrity of membranes, cell walls, etc. in the tissue since electrical impedance decreases when membrane destruction occurs.

Impedance measurements are performed by inserting electrodes into the tissue to be studied and the impedance is measured directly. Certain assumptions must be made in interpreting the results of electrical impedance. The assumption is that biological systems act analogous to an electrical model. In the model the membrane provides a source of capacitance. The destruction of the membrane will produce an effect analogous to the loss of capacitance in the electrical circuit. More sophisticated models can be employed, but the simple model seems to be satisfactory for most studies.

#### VIII. Cold Resistance Project Planning Conference Objectives

The long term objective is to develop high yielding and high quality potatoes with improved frost tolerance. Initial primary emphasis should be placed on: 1) Selection of the most suitable screening technique considering the limitation of personnel and facilities, and 2) Initiate a potato breeding program involving recombinations between S. tuberosum and frost tolerant species.

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## APPENDIX 2

### BREEDING POTATOES FOR FROST TOLERANCE

N. Estrada Ramos

#### 1. Genetic sources

At least sixteen potato wild species have been reported as having high frost tolerance. The following can be mentioned: S. acaule, boliviense, brevicaule, bukasovii, megistacrolobum, santa-rosae, sogarandinum, toralapanum, tuquerrense, vernei, canasense, chomatophilum, commersonii, demissum, multidissectum.

It has been reported also high tolerance in some clones of the five cultivated species. S. andigena (tuberosum), curtilobum, juzepczukii, ajanhuiri and stenotomum.

Resistance is then found in all ploidy levels of the known potato species ( $2n = 24, 36, 48, 60, 72$ ), and indicates that there is a good genetic potential to breed cultivated potatoes with frost tolerance.

Different degrees of resistance have been found in various species and clones but a general division could be made into 3 groups. The first or high resistance group would include probably, S. acaule, S. chomatophilum, S. etuberosum, S. commersonii. The second group or next in resistance may include S. juzepczukii, S. ajanhuiri, demissum, multidissectum and most of the wild frost resistant species.

The third group will include the cultivated species S. curtilobum, stenotomum, andigena. Firbas and Ross (1961), Ross and Rowe (1969) Estrada (1953), Hawkes (1958).

#### 2. Crossability

Crossability has not been a barrier to improve resistance since hybrids between resistant species and good cultivated potato clones have been reported frequently in the literature (Bukasov, Kameraz, Mastenbroek, Blanco and Ubeda, Blomquist and Lauer, Ross and Rowe, Estrada, etc.).

There are difficulties to cross some of the most resistant as S. etuberosum and S. chomatophilum, but others like S. acaule, brevicaule, multidissectum, ajanhuiri, bukasovii may be crossed rather easily.

The crosses using the resistant clones of cultivated species are easy with exception of S. juzepczukii which is highly sterile because it is a triploid, interspecific hybrid, which brings much chromosome imbalance.

### 3. Heritability

Resistance is reported to be inherited in many cases as a dominant factor, specially in crosses using S. acaule (Mastenbroek 1956, Blanco and Ubeda 1966).

In other cases it is reported as a cumulative or as a recessive character.

Vesselovskii (Hudson 1936) obtained acaule x goniocalyx hybrids which survived -5-5°C and could be crossed to S. tuberosum. Hybrids between diploid cultivated species and S. brevicaule, S. vernei and S. multidissectum have been reported resistant to -5°C by Richardson and Estrada (1971). Good resistance was also found by Blomquist and Lauer (1962) in acaule x tuberosum hybrids and by Blanco and Ubeda (1966) in acaule x tuberosum hybrids.

### Gene exchange

Blanco and Ubeda (1966) found that backcrossing to tuberosum employing S. acaule as a frost resistant source showed resistance to -5°C for 3 hours and relatively high tuber yield. This was confirmed by Estrada (1965) and both authors were able to observe quadrivalents in the hybrids ( $F_1$ ) which suggested good possibilities of gene exchange.

The heritability of frost resistance in the hybrids appears to be good according to reports on resistance given by Bukasov, Kameraz, Mastenbroek, Estrada, etc. They have found little linkage between frost reaction and undesirable characters and good tuber type plants can be obtained in the first back-crosses. Estrada (1965).

Inheritance patterns, however, in advanced generations appear complicated. They could be simplified using diploid species according to Ross and Rowe (1965). As far as obtaining varieties with frost resistance, only a limited degree of resistance has been incorporated, Dearborne (1967), Estrada et al (1972).

### 4. Selection systems

Different authors have used various systems to test for resistance. Firbas and Ross (1961) used detached leaves and left seedlings in gauze frames exposed to -2.5°C for 2 hours. Blomquist and Lauer (1962) used detached

leaves and Blanco and Ubeda (1966) used the whole plants in pots. Ross and Rowe (1969) submitted the plants to natural frost conditions in the field (at the end of fall) and Richardson and Estrada (1971) and Alvarado et al (1972) tested plants in growth cabinets, in the field under natural occurring frosts.

Different selection methods thus far used have resulted in some progress but they are still the main limiting factor in a mass program of testing. The main problem is to develop a system as close to natural conditions as possible, and to obtain precise temperature control. Otherwise a mechanism for testing the influence of potentially injurious cold stress without subjecting the plant to cold stress would be useful.

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***Potato Quality***

## CONTENTS

	Page
IN MEMORIUM	307
I INTRODUCTION	311
II PROTEIN - Increasing Quantity and Quality	313
III POTATO QUALITY - Factors other than Protein	315
IV PROCESSED QUALITY OF POTATOES	316
V CHEMICAL ANALYSES	319
VI RECOMMENDATIONS	321
APPENDIX 1 Total Protein Estimation	328
APPENDIX 2 Methionine Bio-Assay	331
APPENDIX 3 Amino Acid Bio-Assay	333
APPENDIX 4 Cystine Analysis	335
APPENDIX 5 Nutritive Value of Potatoes - A Review	337

# IN MEMORIUM

This Report is dedicated to the memory of

Dr. Robert Luescher

who died on March 8, 1974, following a brief illness. The "Genetic Variability of "Available" Methionine Total Protein, Specific Gravity and Others Traits in Tetraploid Potatoes", the subject of Dr. Luescher's thesis (1972), served as the basis for his outstanding contribution to this Planning Conference. The microbiological techniques for assessing the nutritive qualities of the potato were developed through Dr. Luescher's research prior to joining CIP in October, 1973.

His approach to life and to his work can serve as an example to all of us.

## PLANNING CONFERENCE ON POTATO QUALITY

Held at CIP, Lima, November 1973

At the request of Dr. Richard Sawyer, Director General of "El Centro Internacional de la Papa", a Planning Conference was held to examine priorities and recommend specific programmes for the next five years on potato quality.

This document summarizes the discussions and recommendations, indicating research priorities and suggesting sources from whom cooperation might be sought to successfully complete the envisioned goals.

Participants in the Planning Conference were:

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CIP personnel participating in the Planning Conference were:

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Dr. William Roca	Physiologist
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## I INTRODUCTION

The potato is the fourth most important food crop in the world. Per hectare it produces, on average, more dry matter than the legumes and more than any of the cereal crops except maize, which exceeds it by some twenty per cent. Nutritionally the potato is perhaps the most balanced of the major food crops in that it provides calories and nitrogen in proportion to adult human requirements, coupled with a sufficiency of vitamin C and considerable amounts of some B-vitamins.

The potato has the disadvantage in comparison with the cereals in that it is more perishable after harvest. A spectrum of processed products has been developed which now permits the excellent nutritive characteristics of the potato to be retained over relatively long periods of storage. While many processing techniques require sophisticated equipment, some progress is being made in developing procedures which are applicable to small-scale village processing in developing countries.

Prior to the Planning Conference on Potato Quality a number of CIP personnel met to discuss some items of particular interest to them. The proceedings of this meeting were formulated as a series of questions that are indicative of problems confronting CIP in its efforts to improve potato quality. It is informative to consider some of these questions relating to research to improve quality:

### A. Cultural Procedures:

1. Is the phenotypic expression, relative to protein production, best evaluated under experimental conditions with or without the addition of nitrogen fertilizer?
2. What is the availability of nitrogen at zero level addition of fertilizer? Should a standard soil test be used and nitrogen added to some arbitrary level?
3. Which mechanism limits the initial control in protein synthesis: nitrogen uptake and/or translocation?
4. What new equipment do we need for application of fertilizers?
5. Should quality assessment be under conditions appropriate only to developing countries: - no highly specialized breeding program; short days; low-fertilizer availability?



6. Are there cultural practices which may be used by more primitive agriculture to raise protein content?

B. Screening Procedures:

1. What nitrogenous compound (s) do we screen for? What sequence would be used in screening: - total N-dry matter; specific gravity; quality assessment by microorganisms; methionine level; etc. ?
2. What is the most rapid way to screen for protein level and quality in a large number of clones, i.e. 4,000 - 10,000?
3. What genetic sources of diversity should be considered? What is the current status of protein level in potato varieties cultivated in Peru? What is the level in the late blight resistant material available in Toluca, Mexico? What Solanum species are to be examined?
4. Is screening designed to identify genetic sources of high nutritive value at harvest or should screening also include the influence of storage? Is there anything that can be done by genetic manipulation to modify potential changes in storage?
5. Should an arbitrary goal be set regarding the level of protein content to be achieved? Are lower limits for nutritional quality to be established?
6. What is the priority of protein level versus disease resistance?
7. Are levels of antimetabolites such as solanines and phytate to be evaluated during screening?
8. How do we measure progress in a complex breeding program relative to general quality improvement?

While these questions provide an insight into some of the factors to be considered in cultural and screening procedures, similar questions can be raised concerning methods of processing potatoes for storage in developing countries. In addition, detailing precise methods for analyzing potato quality requires a critical evaluation of procedures used in chemical analyses of other food crops as well as an intimate knowledge of the nitrogen and carbohydrate metabolism of the potato plant.

For purposes of organizing the Planning Conference on Potato Quality, the following four areas were designated for discussion:

- I. Protein - increasing quantity and quality. This includes breeding techniques, the effect of fertilizing, seasonal variations, soil type, maturity, nutritive value and other items.
- II. Potato quality factors other than protein. In this section are included the chemical composition of potatoes, their nutritional value and effects of methods of cooking on nutritive value.
- III. Processed quality of potatoes. This includes how the food value of the potato is affected by the various forms of processing.
- IV. Chemical analyses used for determining potato quality.

## II. PROTEIN - INCREASING QUANTITY AND QUALITY

The nitrogen of the potato is combined in many forms of which protein, including the enzyme protein, nucleic acids, free amino acids and amides, and anti-nutritional compounds such as solanidine and its derivatives are of direct relevance to the nutritional quality. Total nitrogen, in that it includes material which is probably of no nutritional significance (such as cell wall protein) or of anti-nutritional significance, cannot be taken to give an estimate of the nutritive value of the tuber, unless it can be shown directly correlated with the nutritionally available nitrogen.

Tuberosum cultivars in Europe and North America are at least as good a source of available nitrogen (based on the amount of potatoes required to maintain nitrogen balance in the adult human male) as of calories. The quality of the protein, with respect to most of the essential amino acids is very good, but there is a deficiency of sulfur - containing amino acids. Taking average values of analyses of European potatoes, the daily requirement (adult 70 - kg male) for most essential amino acids would be met by the consumption of less than one kilo of potatoes and in many cases by little more than 0.5 kilos, although a consumption of about 2.5 kilos would be needed to give the requirement of sulfur-containing amino acids. The daily requirement of calories would be provided by about 3 - 3.5 kilos, depending upon whether one takes the requirement as 2,500 or 3,000 calories.

The variability that has been found just within Tuberosum cultivars indicates that there is a potential for greatly increasing both the content of protein and of methionine in the protein. However, because the potato already contains

protein in quantities as great or greater, relative to the requirements, as those of other nutritionally important constituents (other than vitamin C, of which it is a very important source), breeding for increased protein, or increased methionine, should for utilization in the developed countries be followed only so far as it is compatible with the achievement or retention of other qualities such as disease and insect resistance, productivity, wider adaptability, and improved processing and consumer acceptance characteristics. In the developing countries it is possible that circumstances may exist that will make high protein content preferable to some of the other criteria of selection such as productivity or improved processing characteristics. In this respect it must be admitted that results to date have not shown great promise of achieving a combination of high yield (i. e. fresh weight per hectare), high dry matter and high protein, in that there is sometimes a negative correlation between yield and crude protein, and no correlation between total nitrogen and dry matter. On the other hand, methionine, which can be taken as limiting the value of the potato as a source of protein, shows no negative correlation with yield and there would seem to be some promise of combining adequate yield, medium dry matter (of the order of 20%), and high methionine. Particularly if one extends beyond the *Tuberosum* cultivars, the potential for high protein is much increased and it may be possible to combine a high yield with a protein content or, more importantly, a methionine content, which, though it may be towards the lower limit of the potential range of, say, *S. phureja* is nevertheless higher than the present norm.

With respect to the CIP collection, one objective may eventually be to determine the content of available protein, the amino-acid pattern of the protein and the free amino-acids, of those members of the collection which appear from preliminary analyses to be most promising. It would be inconceivable to perform detailed analyses on the collection as a whole. Preliminary analyses should include dry matter, total nitrogen and protein nitrogen determinations (Appendix I) and could with advantage include determination of the electrophoretic pattern of the protein by acrylamide gel electrophoresis. This is quick and simple and provides a means of "finger-printing" the varieties in the collection (see J. A. Zwartz, 1966, *Eur. Potato J.* 9, 111-128; S. Desborough and S. J. Peloquin, 1968, *A. Potato J.* 45, 220-229; V. Mac and H. Stegemann, 1969, *Hopee-Seyl. Z.* 350, 917-919; R. M. Zacharius et al., 1971, *Am. Potato J.* 48, 57-63). There should be, on selected samples, a microbiological determination of the relative nutritive value with reference to casein. This last could be coupled with estimations of "available" methionine and, preferably, "available" cystine also (Appendices II and IV).

Potatoes are scarcely ever the sole source of nitrogen in the diet, and the supplementary value of potato protein in combination with other food protein should be assessed on a limited amount of material. The "other food protein" should be chosen on the basis of its relevance to the diet of the areas with which

we are primarily concerned - legumes, milk, egg, chicken, fish, pig - meat spring to mind. In this connection the findings of Kofranyi are of importance (1971, Proteins Food Supply Repub. S. Afr. Pap. Int. Symp. 1968, 345-353; 1972, Melsunger Med. Mitt. 46, Suppl. 1, 15-23). Of the natural protein sources he tested, egg protein had the highest biological value, but potato protein was nearly as good, while a mixture of egg protein and potato protein in the ratio of 7:13 had a higher value than either of the constituents, and higher than any other mixtures tested. There are optimal proportions of amino acids in the diet, and any deviation from these proportions results in a decrease in biological value of the protein. A mixture of egg and potato proteins in the above ratio gives a more nearly optimal proportion of the amino acids than either egg or potato protein alone. These findings have relevance to a breeding programme which could be devoted to changing not only the amount but the composition of the protein in the potato.

Prior to the release of breeding material as part of the outreach programme, or of clones for cultivation, the glyco-alkaloid content should always be checked, and material with an undesirably high content discarded.

### III. POTATO QUALITY - FACTORS OTHER THAN PROTEIN

If we are concerned primarily with food value other than protein, the relevant factors are available carbohydrates, vitamin A, vitamin B, vitamin C. The first and last of these are of particular importance in the potato.

The content of available carbohydrates (mainly starch) is usually derived from the total content of dry matter by using a factor which in general, approximates three quarters of the dry matter. Cell wall material, reported as amounting to about 1% of the fresh weight (say 5% of the dry weight), is nutritionally unavailable but may have importance as fibre in the diet. In analysing the main bulk of material for available carbohydrates it would be adequate in the first instance to determine total dry matter and apply the appropriate factor. Supplementary analyses on selected material could with advantage include direct determinations of cell wall material.

The percentage dry matter of mature tubers in commerce ranges from about 17 - 27. Higher values of 30% or thereabouts have been achieved. There is thus scope for increasing the percentage dry matter of the crop from the 22 - 23% usual in Europe and the 20 - 22% common in North America. It must be remembered however, that the factor of importance in potato production is the production of dry matter per hectare and that a negative correlation has been demonstrated between yield and percentage dry matter. Increase in dry matter

content must only be sought if it is accompanied by a less than proportionate decrease in yield. In other words one must concentrate on yield of dry matter per hectare.

Vitamin C is present in the freshly harvested potato in amounts ranging from about 20 - 40 mg per 100 g fresh weight. There is rapid decrease after harvest and a level of about 8 - 10 mg per 100 g is eventually reached. Despite this, the quantities eaten render potatoes one of the most important, perhaps the most important, source of vitamin C in communities in which the potato is a staple foodstuff. Little is known of varietal differences in rates of vitamin C loss in storage or of content at harvest.

It was noted by participants that the potential contribution of the potato to the human requirement for vitamin A could be up to 30% per kg consumed in the case of yellow-fleshed varieties. These contain up to ten-fold the amount present in white-fleshed varieties. Judging from breeding efforts with the tomato and the sweet potato it would appear that the chances are good for increasing even further the vitamin A content of the potato.

Apart from nutritive value, the contents of reducing sugars, citric acid, iron and phenolic substances such chlorogenic acid, are of importance in specific respects - for example, after-cooking non-enzymic blackening of cooked potatoes results from a chlorogenic/iron complex. Citric acid reduces the blackening by chelating the iron and is also of interest as a by-product in, for example, starch factories. Reducing sugars are of over-riding importance in determining the quality of potato chips. Sugar content and content of phenolic substances and the susceptibility to accumulate sugars at low storage temperatures (< 5°C) are all heritable. Internal bruising is of very great importance in the developed countries where potato processing is widespread. This results from enzymic blackening in areas where cell breakage has occurred following impact. The fragility which leads to it appears to be heritable.

#### IV. PROCESSED QUALITY OF POTATOES

Proper storage of potatoes in many parts of the world is very poorly developed or is nonexistent. Along with the development of storages and storage methods it would be well to extend the season of availability of the potato for consumption by preserving the potato in some processed form which could be stored fairly easily and reconstituted or combined with other foodstuffs by uncomplicated methods.

These methods of preservation, most likely some form of dehydration, must be by simple techniques and relatively easily accomplished at the village level. The products should be less perishable than the original potato and also preferably, with little or no loss in nutritional value.

Probably none of the above qualifications is met in the developing countries at present. Ancient methods of preservation such as making the various forms of chuño and related products result in extending the season of utilization of the potato but result in tremendous loss in nutritional value. Perhaps losses as high as 45% protein, 90% loss of sugars and 50% loss of minerals occur in these processes. Perhaps several methods of storing potatoes unprocessed should be considered here also.

#### A. Storage Methods

1. One method which has been used successfully in several areas is to delay harvest of the crop although it has reached complete maturity. In areas where irrigation is utilized the water is withheld so that the potatoes mature at a desirable time.
2. Storage structures: In many areas very simple storage structures would enable the grower to extend the period of marketing or personal utilization of potatoes. This may be in the form of rather thick walled structures of, for example, adobe, mud or heavily insulating materials such as thatch. Where day and night temperature variation is adequate, these structures could be built so that natural cooling of the air and potatoes in the storage could be attained by opening vents or doors at night.

In some countries the necessity for long storage time would be reduced by growing two crops of potatoes in a twelve month period.

3. Potential methods of processing: Dehydration in some form appears to be most likely method to extend the season of utilization of the product under dry climate conditions or where suitable packaging materials are available as moisture barriers. Some of the methods of potential value might involve simple procedures such as the following:
  - a. Whole unpeeled potatoes could be boiled and peeled, and the produce mashed or extruded into rice or ribbon form and dried in an oven to at least 10% moisture. This product could remain in edible form for several months, though not at high tropical temperatures at which excessive caramelization would occur at a moisture content of 10%. Both this and possible browning during drying may be reduced by the addition of, for example, sulphite. This, however, may not be practicable in the areas under consideration in this Conference.

- b. Other forms of extruded products might be considered. Snack foods made from and other grains are processed by forming a doughlike product which is extruded under high pressure into a dry edible form. Perhaps the potato could be processed into a similar form. The potato could be macerated and the pulp added to grain meal or flour, made into dough and extruded under high pressure. This product also would have to be dehydrated to about 10% moisture.
- c. Products made from mixtures of cereal flours and cooked potatoes such as several baked semi-dry crisp dry products known in Scandinavia have good keeping qualities and may be considered for other regions where fuel is not a limiting factor.
- d. Solar drying to produce flakes appears to be a practical processing technique although vitamin A content may be destroyed.

Any practical method of preservation will need to be adaptable to the use of very simple forms of machinery and equipment, preferably that which can be made in the community where it is to be used. Low cost hand operated grinders and macerators are available on the market and some forms of drying ovens could be made on the spot. It would not be difficult to fabricate such items as a press or other similar equipment for extruding any of the products in the form of dough.

Consideration should be given to the possibility of processing products of extended edible life which are made from potatoes in combination with grains. Such products might be "papa pan", noodles, and other extruded products which utilize any of the available grains, flour, meal, etc.

Conditions vary greatly between the countries and areas in which CIP is interested. Eating habits, combination of the foods now consumed, availability of companion foods, etc., are some of these factors. Perhaps some studies should be made in this area, the results of which may describe where greatest emphasis should be placed as to form of processed crop which would be readily accepted.

Any new processed product which is developed should, of course, be investigated as to its nutritional value. This research would closely follow the procedures for determining the nutritive value of raw potatoes as described in another section of this report.

Perhaps it would be well to encourage investigations on processing techniques in some of the institutions in other countries including the outreach countries. Research in this area should be conducted by well trained food technologists. This may best be done through linkage projects with well selected institutions.

## V. CHEMICAL ANALYSES USED FOR DETERMINING POTATO PROTEIN QUALITY

Inherent in considerations of analyses of potato quality are such factors as methods of sampling, components of protein quality to be assessed, analytical techniques, the influence of fertilizers, and the number of clones to be assessed. Discussion was concentrated primarily on the assessment of methods of analyzing protein in screening a large number of clones such as are being accumulated by CIP at La Molina.

- (i) Influence of fertilizer: Since the nitrogenous components of potato tubers are influenced by nitrogen fertilizer, it is important to establish some standard level of nitrogen availability to minimize stress during growth, to provide a basis for comparative analyses among clones, and to permit rational comparisons of clones from season to season. In consideration of the complexities of soil nitrogen availability, with and without amendment by nitrogen fertilizer, and of such variables as assimilation and transport of nitrogen in the plant, it was concluded that an "adequate" level of nitrogen fertilizer should be applied to field plots. The level was to be determined by practical guidance and in such manner as reliable soil analyses might dictate. Clearly, nitrogen fertilizer amendment was considered essential, to be quantitatively broadcast prior to planting, but careful thought should be given to the level, as effects of this on the content and proportion of essential amino acids in the protein have been reported (B. Mica, 1971, Potato Res. 14, 19-28).
- (ii) Methods of sampling: In common with many other factors that were discussed, it is difficult precisely to delimit sampling methods. In evaluating possibly more than 4,000 clones a rapid method is obviously imperative. Since clones may have different growth periods to maturity, clones should be harvested immediately following the death of the foliage. Thus different clones would be harvested at their approximate mature state as determined by weekly inspections. A succession of different clones would be processed in an orderly fashion. At harvest, tissue samples would be



obtained by non-destructive removal of longitudinal wedges from apex to stem end and to a depth to the center of a tuber. These fresh samples would be immobilized by immersion in liquid nitrogen, or by freeze-drying, or preferably by placing in hot 70-80% alcohol. It is suggested that samples be powdered following freeze-drying and, when possible, standard amounts of powder of known moisture content be taken for analyses.

The possibility of expressing juice from cylinders of tuber, or some other configuration of tuber tissue may have merit in sampling for protein analyses. Early results have indicated that this technique should be further refined as it lends itself to evaluation of soluble proteins by means of refractometric determinations. Sucrose units, expressed as a protein index, correlated very favorably with proteins determined by the Lowry method; the per cent protein refractometer index versus per cent protein by Lowry had a value of  $r = 0.83$  (Figure 1). It is proposed that sampling and protein evaluation by the above procedures might be further investigated through an appropriate linkage project.

- (iii) Analytical techniques: During prolonged discussions of techniques to evaluate selected nitrogen-containing components of potatoes it became apparent that methods used in evaluating protein fractions of cereal seeds were not directly applicable to evaluating protein in a modified stem. The type of proteinaceous substance to be evaluated, the importance of type and source of the amino acid fraction, the method of analyses, and finally, the end point desired in protein assessment were considered.

The following components of tuber protein and methods of analyses were discussed:

1. Crude "protein" or nitrogen  $\times 6.25$  (or 7.5)
2. Peptides
3. Free amino acids
4. Dye binding capacity of proteins
5. Soluble protein
6. Bound protein
7. Net protein content (Per cent protein  $\times 6.25 \times$  Digestibility  $\times$  Biological value) = Relative Nutritive Value

The following tests for determining protein in routine screening were considered the most appropriate:

1. Determine Specific Gravity of a sample.
2. Determine crude "protein" and true protein (by alcohol precipitation) on all samples, using micro-Kjeldahl technique.

3. Determine digestibility and biological value of component amino acids by micro-biological assay, on selected samples.

Other methods of assessing various peptide or amino acid components were rejected. However, protein assessment, as well as starch, lipid and water determinations by means of the Neotec instrument have the merit of permitting extremely rapid quantitative measurements. This approach for assessing total protein as a screening technique should be pursued. In addition, the removal of soluble protein, by acidified alcohol precipitation, prior to micro-Kjeldahl determinations, should be examined.

In screening for protein it is recommended that clones exhibiting less than ten per cent crude "protein" in the dry matter, as determined by micro-Kjeldahl, be eliminated from further protein evaluation. The expression of protein yield as a function of total solids produced per hectare is to be encouraged.

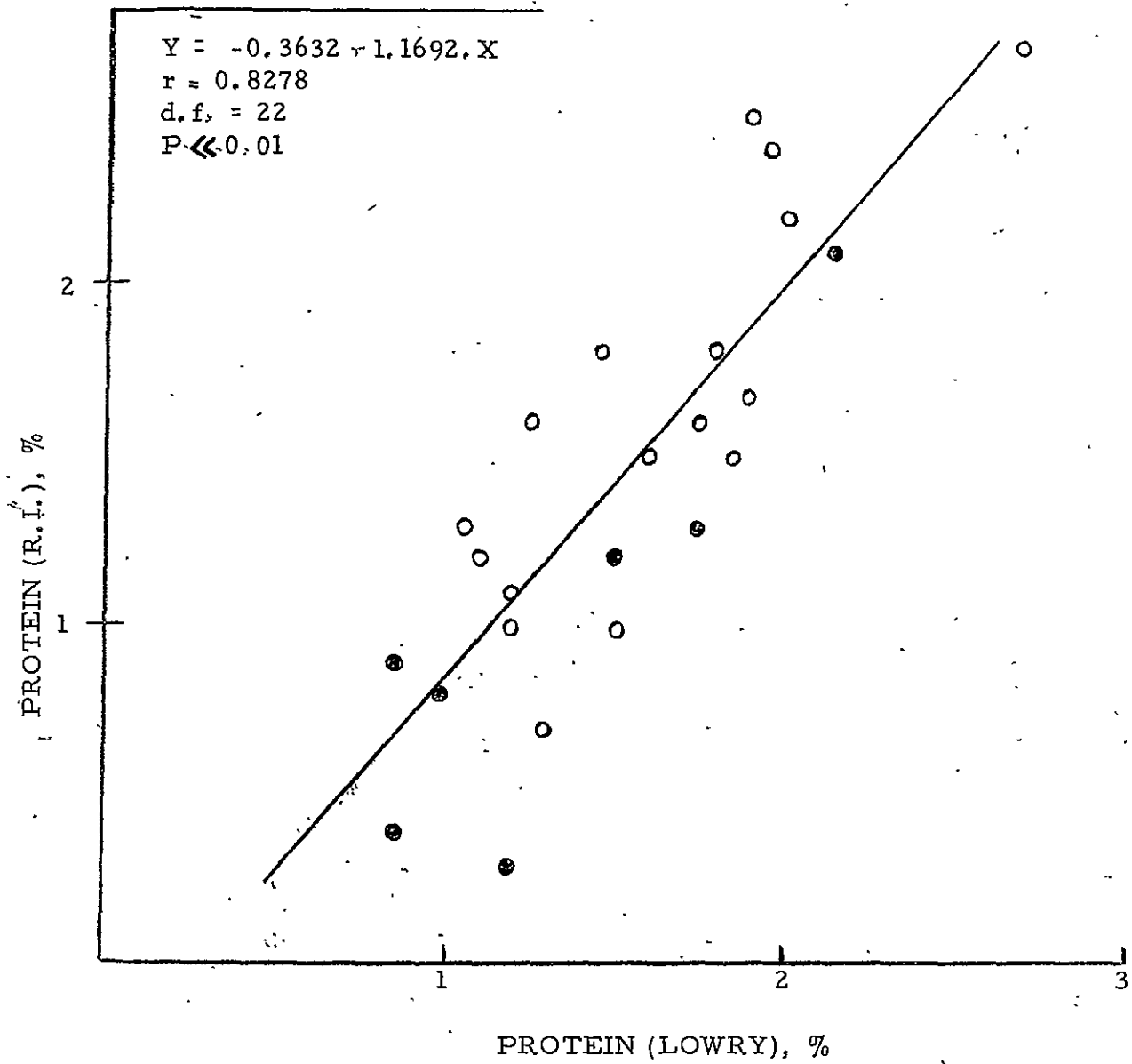
The necessity of evaluating potato protein in biological terms, in addition to purely chemical assessment deserves special emphasis: In a basic screening program the relative nutritive value with reference to casein can be determined by total growth of the bacterium, Streptococcus zymogenes. During this screening "available" methionine can also be assayed with S. zymogenes. If desired, "available" cystine can hopefully be assayed by a bioassay technique using the bacterium Clostridium welchii. The protozoan, Tetrahymena pyriformis may be used in secondary evaluations, this organism exhibiting specific requirements for the ten amino acids generally regarded as essential for the growth of man.

## VI. RECOMMENDATIONS

The techniques involved in making crosses to improve the nutritional quality of potatoes are not considered in Recommendations. But it is stressed that data derived from chemical and biological evaluation of nutritional quality are to provide the basis for selecting parental material. It was however, emphasized repeatedly during the Conference that disease and insect resistance has priority over other properties in making crosses and selections particularly when these are destined for countries where specific diseases are a problem. Also, high dry matter per hectare has priority over a high content of protein.

Emphasis throughout the Conference was placed on protein quality and methods of evaluating quality. The quantitative aspects of protein, carbohydrates and vitamins were also considered. It was generally conceded that it was not difficult to obtain adequate levels of carbohydrates, that a minimum "cut off" for protein be established at 10% crude "protein", that a sufficiency of vitamin

Fig. 1 Relationship between Refractive Index difference and protein as determined by the Lowry method



C was normally present and that vitamin A content could be improved. Attention should be directed to levels of anti-nutritional factors - phytate perhaps, but particularly the glyco-alkaloids. Material with anti-nutritional factors beyond accepted tolerance limits should not be released to countries which do not possess adequate facilities to assess them.

It was also recognized that analytical methods suitable for evaluating the protein content of potatoes are not precisely defined. Thus, despite the urgent need of providing material for early release to selected countries, it is imperative that research be initiated immediately, preferably by appropriate contract projects, to evaluate analytical techniques to separate, identify and quantify the various nitrogenous compounds found in potato tissue.

Promising techniques are being developed for processing potatoes in developing countries to provide nutritious products with good keeping qualities. Dr. A. Bacigalupo, of the National Agrarian University has developed a number of products which have potential for village-type, labor-intensive processing. CIP does not have a staff nutritionist to evaluate potatoes and potato products by means of rat and human assays and to assess cultural food preferences in developing countries. Such value judgments are probably best obtained through linkage affiliations.

The quality of storage facilities has a direct influence in maintaining the nutritive status of potatoes. Dr. Max Milner has proposed the no-cost services of "VITA" - Volunteers for International Technical Assistance - to assist in innovative design of potato storages in developing countries. This group of Volunteers are professional engineers in the employment of General Electric in Schenectady, N. Y.

Specific project recommendations have been divided into those most suitably undertaken by CIP personnel and those most efficiently handled through linkage or contract arrangements.

A. Recommendations - CIP Projects

1. Broad-scale screening:

- a) Prior to planting, soil is to be finely prepared and fertilizer is to be uniformly broadcast. Fertilizer type and rate are to be determined in conjunction with soil analyses. It is recommended that fertilizer amendments be standardized in a manner to minimize yield and quality variations from year to year.

- b) Death of foliage, determined by weekly inspection, is to be considered as an indicator of maturity and is recommended as the time to harvest. Yield data are to be recorded.
- c) Specific gravity is to be determined on clonal samples by weighing in air and in water. Graded saline solutions may be used as an auxiliary method for small tuber samples.
- d) Tubers are to be sampled by the non-destructive removal of longitudinal wedges from apex to stem end to a depth to the center of a tuber. A blend of sub-samples of a clone, including tubers of various sizes, is suggested. Flesh color is to be assessed by reference to a color chart.
- e) Fresh tuber samples are to be immobilized as soon as possible after harvest by low-temperature freezing (e.g. "dry ice") and then freeze dried. Fresh and dry weights of each sample are to be recorded. Samples will be powdered after drying and stored in a refrigerator.

Study Period: Basically this is to be a continuing program throughout a five-year period. Commencing in 1974, 40 advanced clones with potential for Outreach distribution and grown in four environments, will be screened. Thus, a total of approximately 1860 ( $4 \times 40 + 200 + 1500$ ) clones will be screened in 1974. In 1975 and 1976 selected clones from 1974 will be screened again along with additional clones from the germ plasma collection to give a quota of approximately 3000 clones in each year. A review of progress will be made annually, with a more thorough overall review in 1976.

Co-ordinators: Dr. R. Lüscher and Dr. P.R. Rowe

## II. Quality Screening Sequence:

- a) Nitrogen determinations by micro-Kjeldahl  $\times 6.25$  are to be made on each sample of freeze-dried tuber powder. The efficiency of extraction of soluble, non-protein nitrogen by acidified ethanol as a pre-treatment before Kjeldahl determinations is to be evaluated (Appendix I).
- b) Relative Nutritive Value is to be determined. RNV is equal to Kjeldahl N  $\times 6.25$  digestible protein  $\times$  biological value of the amino acids. It is to be determined following microbiological assays with Streptococcus zymogenes and/or Tetrahymena pyriformis (Appendix II and III).

Dry matter yield is to be expressed in kgs./hectare.

Study Period: As per Recommendations - CIP Projects, Section 1.

Co-ordinator: Dr. R. Lüscher

III. Specific evaluation of protein quality:

- a) It is recommended that selection and breeding of clones of high methionine content be given priority over increase of protein content. Initially a biological assay of methionine is preferred. Analysis of specific amino acids in tubers by Amino Acid Analyzer is recommended in specifically selected clones. Key amino acids to be evaluated are methionine, cystine and possibly proline. Limited data from selected families show that methionine is not highly correlated with protein content ( $r = .69$ ). Proline is correlated ( $r = .84$ ) with protein and would be one amino acid of choice for screening when suitable rapid quantitative methods are available for its determination.

It is recommended that the Analyzer presently available in La Molina be repaired and used for this purpose.

(See Appendix III regarding cystine).

Study Period: Methionine and cystine content to be routinely assessed by microbiological assay commencing in 1974. Progress to be evaluated annually with detailed review in 1976. Amino acid analysis to be considered in 1976.

Co-ordinators: Dr. R. Lüscher, Dr. K. Sayre, Dr. W. Roca

- b) It is recommended that selected parents, or advanced clones prior to release, be assayed for general glyco-alkaloid content.

(There are no rapid methods presently available to assay glyco-alkaloids. A new method which measures the nitrogen present, rather than double bonds, should be forthcoming in the near future according to Dr. E. A. Talley). The following background references from Chemical Abstracts are pertinent: 37 4087 (1943), 38 40547 (1944), 55 22351 b and h (1961), 59 1709 g (1963) - describes a very useful ThC system, 60 15943 d (1964), 72 87188 (1970), 77 149672 (1972), 77 149774 (1972).

Study Period: Evaluation of methods should commence in 1974. Routine evaluation of selected materials should continue at least until 1976 and progress and the need for continuation assessed.

Co-ordinator: Dr. K. Sayre

## B. Recommendations - Contract Projects

### Priority 1

- a) It is recommended that methods of extracting and determining soluble tuber protein be investigated. The following techniques are to be examined and compared:
  - (i) Extraction of freeze-dried powder with 80% ethanol, 10% TCA, and 1% picric acid, as well as other concentrations of these solutions;
  - (ii) Dialysis in running tap water as per Fitzpatrick et al (Am. Potato J. 46: 273-286, 1969).
  - (iii) Evaluation of technique of expressing fresh juice as well as reconstituted freeze-dried powder and determining soluble protein by refractometer.

Study Period: Two years duration commencing in 1974.

- b) Rat bio-assays to assess protein quality relative to microbiological evaluations are recommended. It is proposed that some comparative tests to be made as soon as possible in order to verify relative nutritional ratings determined with S. zymogenes. It is recommended that 6 - 10 replicate rats be used in each trial.

Study Period: Commence in late 1974 or early 1975 and continue for 4 or 5 trials.

### Priority 2

- a) Assessment of procedures to evaluate such glyco-alkaloids as alpha- and beta-chaconine, alpha-solanine, demissine, leptines and solamarines as well as phytate.
- b) Assessment of vitamin C loss during storage, relative to varieties; influence of freeze-drying on vitamin C loss during storage.

The same contract project may also be concerned with rapid techniques assessment.

- c) Progress would be more rapid if basic genetic information is available in order to effectively breed for desirable traits within Solanum. To establish linkage groups or chromosome maps reasonably efficient phenotypes reflecting specific genotypes need to be used. Biochemical gene

markers in conjunction with trisomes would be an example of a fairly rapid system of gene mapping. Potato is one of the major food crops which lacks essential genetic information required in any breeding program.

- d) Inhibitor proteins may account for up to 10% of the soluble protein in tubers. The uniqueness of this group of proteins would make them very suitable for specific inheritance studies.

One basic question is: Do selections high in protein contain increased amounts of these inhibitors? The inhibitor proteins would be of great value in studies of protein synthesis since they can be readily identified.

#### C. Recommendations - Fundamental Research

- a) The panel recognizes the importance of fundamental research as an underpinning of applied research and in particular of the contemplated attempts of creating high protein and high methionine potatoes. It therefore, recommends that the Center actively encourages and endorses research activities in the following two areas as considered essential to success of its mission:
- b) Cytogenetic and biochemical investigations with the aim of identifying gene markers and the establishment of a chromosome map of the potato.
- c) Biochemical investigations on the nature of storage proteins of the potato, the factors that influence their production and deposition, and the biological function of the individual constituents.



## APPENDIX 1

### PROCEDURES FOR TOTAL PROTEIN ESTIMATION

### IN POTATO TUBERS BY KJELDAHL TECHNIQUE

P. H. Li

#### I. Sample Preparation:

1. Wash tubers, weigh, and store in the cold storage room or refrigerator if not immediately for further treatment.
2. Remove longitudinal tuber wedge(s), weigh, and freeze immediately prior to freeze-drying.
3. Determine the dry weight after freeze-drying.
4. Grind into 60-mesh size powder, and store in a freezer until analysis.

#### II. Pretreatment of sample:

Data collected from CIP's samples indicate that percentage of non-protein nitrogen can be varied from 35 to 63%. Removal of non-protein nitrogen is, therefore, a necessary step in order to obtain a meaningful estimation of total protein by Kjeldahl technique in potato tubers.

1. Weigh exactly 1 gram of dry-powder into a 100 ml of Erlenmeyer flask, add 50 ml of 80% alcohol, and stop with a rubber stopper.
2. Shake the sample on a Forma Model 4537 shaker for at least 25 minutes at its highest speed.
3. While shaking, prepare an appropriate number of 150 ml of beakers for filtration with weighted filter paper.
4. Quantitatively transfer the slurry onto the filter paper. Wash the glass-ware, and then wash the residue with an appropriate amount of 80% alcohol until the filtrate is about 100 ml of volume.
5. Dry the residue with the weighed filter paper in an oven at 70°C for 24 hr. and weigh the total dry wt. of residue after drying.

6. Weigh an exact 100 mg residue for Kjeldahl N determination - Total protein nitrogen.
7. Convert the total protein nitrogen from residue to the dry weight of sample basis. Time the protein factor - Total true protein content.

### III Kjeldahl Determination:

#### 1. Digestion:

- a) Weigh exactly 100 mg or more of sample, and add 100 mg of catalyzer (Selenium mixture) into a Kjeldahl flask.
- b) Add 2 ml of the digestive solution ( $H_2SO_4$ ), and digest on the heater. Digestion will last about 30-40 min.

#### 2. Distillation:

- a) Add 20 ml of the "Indicator-Reagent" (Boric acid plus indicator) into a 50 ml Erlenmeyer flask, and place it under the exit of the distilling apparatus.
- b) Quickly pour the digested solution into the upper container of the distillator, rinse Kjeldahl flask with distilled water and pour into the same container.
- c) Open the stopcock and let the solution drain into the lower container of the distillator drop by drop.
- d) Close the stopcock after all the solution has been transferred into the container and then add an appropriate amount of NaOH to the container.
- e) Open the stopcock again and let solution mix slowly with digested solution; as soon as mixture turns violet, the stopcock should be closed immediately.
- f) The above mixture will be distilled to allow the  $NH_3$  to be absorbed in the Indicator-Reagent. Notice changing color (greenish). Wait 3 more minutes to allow all of the  $NH_3$  to be completely absorbed.

#### 3. Titration:

a) Remove Erlenmeyer flask and titrate with 0.1 N  $\text{H}_2\text{SO}_4$  until to the end point (violet color).

b) Record ml of  $\text{H}_2\text{SO}_4$  used for titration.

4. Calculation:

Use reference from general chemistry for N calculation after titration.

5. Reagents:

a)  $\text{H}_2\text{SO}_4$  -  $\text{K}_2\text{SO}_4$

1) Dissolve 25 g. potassium sulfate in a portion of concentrated  $\text{H}_2\text{SO}_4$ .

2) Prepare a saturated cupric sulfate solution. Remove 25 ml of  $\text{CuSO}_4$  solution and add into 1).

3) Add 10 g. of Mercuric oxide yellow to 1).

4) Make to 1000 ml with con.  $\text{H}_2\text{SO}_4$ .

b) NaOH - 1000 g of NaOH in 1000 ml of distilled water (with care) and then add 1 g. of phenolphthalein.

c) Indicator Reagent:

1) Dissolve 100 mg methylene blue in about 20 ml of 95% EtOH.

2) Dissolve 180 mg of methyl red in about 20 ml of 95% EtOH.

3) Mix 1) and 2) and then make to 1000 ml with 95% EtOH.

4) Take 1 ml of the solution from 3) and mix with 1000 ml of 20% boric acid. (20 g. of boric acid plus 100 ml of  $\text{H}_2\text{O}$ ).

## APPENDIX 2

### MICROBIOLOGICAL BIO-ASSAY TO MEASURE "RELATIVE NUTRITIVE VALUE" AND "AVAILABLE" METHIONINE IN POTATOES

R. Lüscher

Summarized, Streptococcus zymogenes needs broadly the same amino acids as the growing rat. It is powerfully proteolytic and grows quickly with an adequate intact protein as the main source of nitrogen. Although lysine and serine are not indispensable, the absence of these in the growth medium restricts growth severely. Cystine "spares" methionine, in the sense that omission of cystine from the test medium increases the requirement for methionine by about 15%.

Streptococcus zymogenes has successfully been used to provide an estimation of protein quality. For 16 different food proteins, growth of S. zymogenes (=Relative Nutritive Value with reference to casein) correlated highly ( $r = 0.9$ ) with the net protein utilization (NPU) value determined with the rat. With another set of 17 whale-meat meals, fish meals and meat, a similar correlation between growth of S. zymogenes and NPU values determined on rats was calculated ( $r = 0.81$ ). Net protein utilization is equal to biological value (BV) X digestibility of the protein divided by 100. The fact that growth of S. zymogenes correlates with NPU values suggests that a bio-assay, with this proteolytic bacterium, can give an estimation for both BV and digestibility of the potato protein. "Available" methionine assayed with S. zymogenes agrees very well with chick results ( $r = 0.97$ ). When heat damaged whale-meat meals were fed to rats and analyzed for total and "available" methionine, the Net Protein Utilization (NPU) values of the rat correlated  $r = 0.57$  with total methionine (determined after acid hydrolysis) and as much as  $r = 0.92$  with "available" methionine determined with S. zymogenes and enzymatic digestion. It is obvious from these results that the method involving S. zymogenes and enzymatic digestion is capable of measuring the biological "availability" of methionine in foodstuffs.

#### 1. Method of Analysis:

1. Samples containing 50 mg of crude protein ( $N \times 6.25$ ) are weighed out into 100 ml bottles and suspended in 20 ml citrate cyanide buffer.
2. PH is adjusted to 7.2, the bottles are placed into a water bath at 55°C. Two ml of 4% (W/V) crude papain are added and incubated for 3 hours.

3. The samples are filtered through a Whatman N°4 filtered to remove starch, the pH adjusted to 7.2 and the volume is made up to 100 ml.
4. Two ml of the digests are added to each of 4 test tubes. To two test tubes, a medium containing vitamins, glucose salts, bases and all amino acids with the exception of methionine is added ("available" methionine).
5. To the two other test tubes the same medium but with the omission of the amino acids, is added (relative nutritive value).
6. After sterilization 1 drop of an actively growing culture of S. zymo-  
gene is added to each test tube, including the methionine and casein standards.
7. When the incubation is finished (40 h) the optical density of each test tube is measured at 580 nm with the aid of a flow through cell.

## II. Bio-assays with Bacteria are Advantageous Because:

1. Less than 1 g of dry matter is required.
2. The assay takes just 2 days.  
Rat assays need several thousand times more dry matter and last 2-4 weeks.
3. Only a moderate investment in laboratory equipment is necessary.
4. Bacterial assays can well be adapted for routine analysis of a large number of samples.

### APPENDIX 3

#### AMINO ACID ASSAY USING THE PROTOZOON

#### TETRAHYMENA PYRIFORMIS W

R. Lüscher

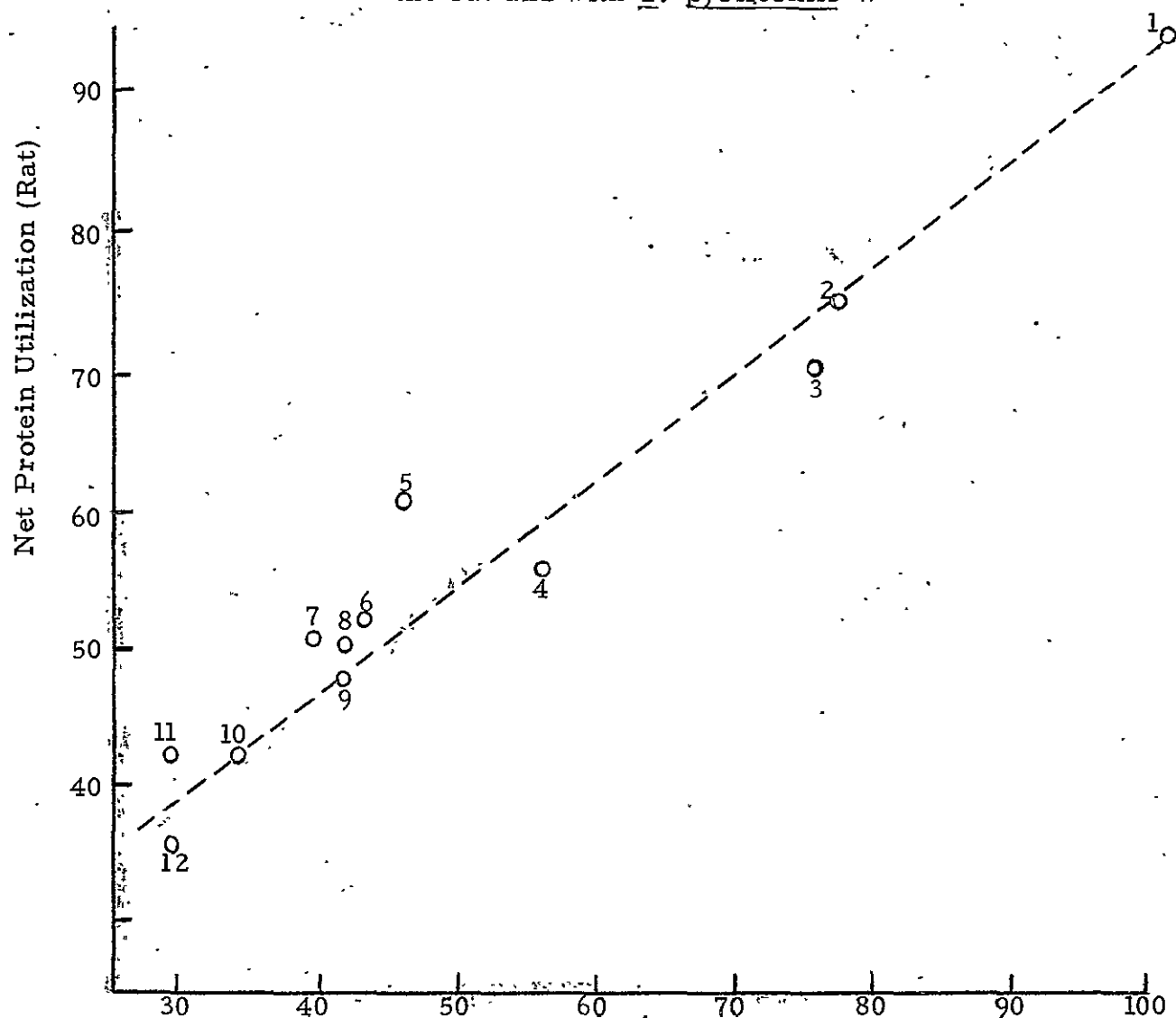
Tetrahymena pyriformis W. is, as a protozoan, a more highly sophisticated micro-organism than Streptococcus zymogenes.

T. pyriformis W. requires absolutely: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, the ten amino acids generally regarded as essential for the growth of higher animals.

1. Procedure:

1. A sample containing 3 mg crude protein (Kjeldahl N x 6.25) is weighed into a 125 ml Erlenmeyer flask. Then various solutions containing glucose, vitamins, purines, pyrimidines and salts are added into the Erlenmeyer flask.
2. Sterilization of the flasks is followed by adding 3 drops of an actively growing T. pyriformis culture to each flask.
3. After an incubation time of 4 days at 25°C, growth of the protozoan is measured by counting a 1 ml sample in a haemocytometer under the microscope. The total number of organisms/ml of medium is directly proportional to the NPU value of the protein.

Fig. 2 Comparison of protein nutritive values obtained with the rat and with T. pyriformis W



Relative nutritive value of T. pyriformis W  
with reference to egg protein 100

- |                     |                         |
|---------------------|-------------------------|
| 1. Whole egg powder | 7. Groundnut oil meal   |
| 2. Skim milk powder | 8. Hope gram (whole)    |
| 3. Casein           | 9. Green gram dhal      |
| 4. Soybean (whole)  | 10. Peas, black variety |
| 5. Copra oil meal   | 11. Aconite beans       |
| 6. Bengal gram dhal | 12. Lentil dhal         |

## APPENDIX 4

### CYSTINE ANALYSES IN POTATOES

R. Lüscher

Dietary cystine reduces the methionine requirement in mammals. It is therefore important that methionine and cystine analyses are performed. The traditional analyses of cystine involving acid hydrolysis is not suitable for potato samples, because of the destruction of cystine due to the presence of large amounts of carbohydrates. However, there are three alternative methods available worthy to evaluate for suitability:

- I. The use of proteolytic strain of Clostridium perfringens. This strain was used to assay for cystine before the automatic ion-exchange chromatograph was introduced. The fact that it is vigorously proteolytic opens the possibility that it could be grown on the enzymatic digest already prepared for the analysis of "available" methionine. Thus, we would measure "available" cystine with this procedure. Under the conditions of the experiments there is no formation of a toxin (lecithinase) or any of the other known toxins or exoenzymes (hyaluronidase, O toxin, gelatinase) in this medium. However, this organism does not lose its ability to form toxins when grown under conditions suitable for toxin production.
- II. Estimation of the sulfur amino acids by a short ion-exchange column method. In this method the samples are first oxidized with performic acid and then hydrolysed. Cysteic acid, methionine sulfone and lysine can be eluted with a buffer. If a fraction collector, a column and a spectrophotometer are available, this procedure should not cause problems. Pertinent references in Chemical Abstracts include: 67 72481, 72486 (1967), 71 861 (1969), 72 28806, 51612 (1970), 73 13176, 33923, 51613 (1970), 74 38993 (1971), 75 137384 (1971), 76 1500, 1501, 1505, 12861, 31983, 31984, 57807, 57813 (1972), 77 18461, 45080, 137043 (1972), 78 68909 (1973), 79 50577, 63355, 63356, 89047 (1973).
- III. Method of N. Taniguchi  
  
This method is simple and accurate and has two steps: (1) conversion to zinc sulfide from cysteine or/and cystine in protein by treatment of a pH 9.5, 0.6% zinc hydroxide suspension at 100°C for 48-96 hours. (2) Colorimetric determination of hydrogen sulfide obtained by acidification of zinc



sulfide. Methionine and cystine synthesis are interrelated. It is therefore important that clones high in methionine are analysed for cystine, too, in order to avoid clones in which a higher methionine value is achieved at the cost of cystine.

## APPENDIX 5

### "NUTRITIVE VALUE OF POTATOES, A REVIEW"

Ora Smith

#### I. PROTEIN - INCREASING QUANTITY AND QUALITY

The principal objective of research in this field is to increase the nutritional value of potatoes by increasing the amount and quality of potato proteins. Interest in the proteins of the potato has increased markedly recently because of the high biological value of potato protein and its potentially high yields of protein per unit area of land. The possibilities of increasing the protein production of potatoes by plant breeding and growing techniques have been widely studied (Fitzpatrick et al 1969; Talley et al 1970; Mica 1971; Westerlind 1971; Varis 1973 and many others). Attention has been paid to proteins in the potato also because of the deleterious effects they often have on the quality of table potatoes and especially on some processed forms (Findlen 1960; Smith and Treadway 1960; Fitzpatrick and Porter 1966; Varis 1970 and others).

The carbohydrate to protein ratio in potatoes is relatively high. It has been shown, however, that the potato can serve as the sole source of nitrogen for humans. Kon and Klein (1928) showed that a man and woman were maintained in good health for 167 days on such a diet by the daily consumption of 1680 and 1120 grams of potatoes, respectively. By increasing the protein content of the potato, it would be greatly improved as a food in many areas of the world.

Chick and Slack (1949) also showed that, unlike many foods, the non-protein nitrogen of the potato has considerable nutritional value, as it consists largely of free amino acids a factor which complements the potato protein nutritionally. The high starch to protein ratio requires a high caloric intake to furnish the daily requirement of protein. More protein per calorie could be supplied if the protein content were increased.

According to Kofranyi and Jekot (1965) the nutritional value of potato protein is as good as or better than whole egg, and better than beef, tuna, whole milk, wheat flour, corn, rice, soybean and kidney bean protein. A mixture of 35 percent whole egg and 65 percent potato gave the lowest nitrogen intake for maintaining a nitrogen balance ever found by these authors.

The nutritional or biological value of protein in potatoes is rather high. Biological value of 258 German samples of potatoes ranged from 61.08 to 88.92 (Schuphan 1959). Potato protein contains substantially more of all the essential amino acids except histidine than that of whole wheat. The amount of lysine in potatoes is similar to that in a typical animal protein (Hughes 1958). Choudhuri et al (1963) found that the potato protein content per 100 gm of raw, cooked, baked, fried and canned potato on a fresh weight basis is 1.96, 1.93, 2.43, 3.73 and 1.6 percent respectively.

In rat feeding experiments Chang and Avery (1969) found that the nutritive value of potato protein was superior to that of rice. Weight gains and protein efficiency ratios were higher in those rats fed the potato diet. The fat concentration in the liver also was significantly lower in the animals fed the potato diet.

MacGillivray and Bosely (1962) claim that potatoes produce more essential amino acids per acre than milk, oats, beef or lamb.

It is well known that the level of soil fertility and nutrient availability affect the protein content of potatoes. Commercial varieties of potatoes grown under the same environmental conditions also vary in protein content. The potential of breeding potato varieties of higher protein content than those presently available is great.

An increase in the dry matter of a tuber is accompanied by a comparable increase in the starch content. The non-starch solids are relatively constant over a wide range of total solids variation. Burton (1948) and others have reported this constant to be about 6 percent. Houghland (1966), however, showed that this figure is not constant between varieties varying considerably in dry matter and starch content.

#### A. Breeding

Since total nitrogen content of tuber ranges from 1.4-2.8 percent of the dry weight and protein nitrogen content rarely exceeds one half of this value, breeding for an increase in dry matter content probably would not be very successful in increasing the protein content of potatoes. Peare and Thompson (1973) found that with 16 cultivars grown in 10 states percent protein was inversely related to the percent of total solids in the tubers. The highest total nitrogen, soluble N and insoluble N, content on a dry basis is found in potatoes with the lowest solids (Talley et al 1961; Talley et al 1964; Talley and Porter 1970; Talley et al 1970).

The nutritional value of a lot of potatoes as measured by total, soluble and insoluble N is the same from those of high, medium and low solids content (Talley et al 1961). There is an inverse relationship between solids content and nitrogen values (total, soluble and insoluble nitrogen) when calculated on a moisture free basis (Fitzpatrick et al 1964). The relationship of insoluble nitrogen to total nitrogen remains fairly constant, regardless of the solids content of the potatoes with ratios varying from 0.39 to 0.43.

The content of individual amino acids on the fresh weight basis also is essentially constant with respect to specific gravity of the potatoes. On the dry basis, however, significant differences in most instances were found between levels of specific gravity groups. Proline content increases with length of storage and especially when the tubers sprout. The changes in alanine are in almost the reverse order (Talley et al 1964).

Fitzpatrick et al (1969) report that the percentage of total nitrogen on a dry weight basis for 83 seedling samples and selections grown in Maine and Idaho, was higher in low solids potatoes than in those of high solids. Calculation for total solids vs protein nitrogen, respectively indicate a direct correlation with the total nitrogen data. On both a fresh and a dry basis, an increase in protein nitrogen results in an increase in the ratio of protein to non-protein nitrogen. The authors state that since a low ratio of starch to protein, or a high energy percentage, indicates a high protein content relative to the starch present, the aim of any breeding experiments should be toward a low ratio and, when combined with a relatively high total solids, a nutritious and productive variety should result. They state further that in samples such as those with which they worked, and probably others, rests a potential for the development of a potato variety containing increased nitrogenous constituents, both in absolute amounts and in amounts relative to the total solids, or better, to the starch content. The goal of future work is to ascertain how these differences are transmitted to the progeny. The present work indicates that there are varietal differences, and later work must determine their inheritance pattern. The stability of the protein content in high protein selections must be tested in different environments. This should be followed by an examination of the high protein selections to determine whether the presently desirable attributes needed in cooking, processing, etc., will be retained.

Sanford et al (1971) reported on the effectiveness of selection for tuber total nitrogen in a tetraploid breeding population. Offspring total N ranged from 0.20-0.50 percent (crude protein percent of 1.25-3.13) in 1968 and from 0.20-0.40 percent that within the tested population, genetic variability exists for total N content of sufficient magnitude to allow improvement by selection.

Desborough and Weiser (1972) determined the soluble and total protein relative to tuber protein inheritance in six diploid and tetraploid Phureja-haploid Tuberosum families. The relative amounts of tuber protein were increased substantially in the first generation of selection. Total protein and soluble protein appeared to be directly influenced by ploidy level and growing location. Their data indicate that the diploid selections have as much or more potential than the tetraploids for a selection program to increase protein. The authors plan an expanded survey of diverse germ plasm in an attempt to find other sources of high tuber protein. Studies of the heritability of tuber protein are in progress. They also plan to compare the amino acids in tubers high in protein, specifically those that are limiting in human nutrition. Their future studies also will consider the effects of increased protein on various cooking and flavor qualities.

Kaldy (1971) and Kaldy and Markakis (1972) determined 18 amino acids quantitatively in Russet Burbank and five clonal selections. Protein scores in the sulfur-containing amino acids for Russet Burbank and the five clones were 73, 78, 60, 62, 73 and 68 respectively. Methionine was the limiting amino acid in all samples.

Kaldy et al (1972) measured the protein content of 21 varieties by the Kjeldahl N determination and by the binding of the dye Orange G. They found a linear relation between the N content and bound dye. The correlation coefficient was +0.9827.

B. Seasonal variations

Yield of protein may vary from season to season depending upon weather conditions. Although Talley et al (1970) found irregular variation in tuber protein content due to weather variations between years, tuber yield apparently has a larger effect on the total protein yield than does the protein content of our present varieties.

C. Soil type

Soil type also influences total protein yield. On peat soils (Varis 1973) tuber yields were high and tuber protein content also was high apparently because of the higher content of nitrogen in the soil.

D. Maturity of potatoes when harvested

Early harvesting results in reduced tuber yield and lower protein content and thus lower protein yield (Varis 1973). Protein continues to move into tubers to the end of the growing season, thus the more mature they are, the higher the protein content.

E. Nutrition of the potato

Fertilizer treatments affect both tuber and protein yields as well as protein content. Varis (1973) obtained lowest tuber yields, lowest protein content and lowest protein yields from unfertilized areas and from those fertilized with farmyard manure. The presence of a sufficient amount of N in the soil seems to be necessary to obtain large protein yields. High N application (150 kg/ha) greatly increased protein content and thus the protein yield. Similar results have been obtained by Swiniarski and Ladenberger (1970) in Poland; Westerlind (1971) in Finland and others.

The application of KCl as the source of potassium compared with  $K_2SO_4$  reduced the protein content and through that, the protein yield as well (Varis 1973). By increasing the application of N from 36 to 336 lbs. per acre crude protein content of potato was increased from 9.5 to 12.9 percent and true protein content from 3.8 to 5.4 percent (Wilcos and Hoff 1970). The amino acid pool in the tubers almost doubled by such increases in N applied (Hoff et al 1971). Increases in individual amino acids ranged from none (tyrosine) to 2.7 fold (glutamic acid + glutamine). Lysine and methionine increased with increasing N applied but the relative proportions of lysine remained unchanged and methionine decreased.

With N applications up to 184 lbs. per acre the essential amino acid content slightly increases, more noticeably with leucine, isoleucine and arginine, less with lysine and phenylalanine, while histidine was only slightly affected (Schuphan 1959).

In Poland Loginow and Klupczynski (1969) found that increasing amounts of N applied resulted in a proportional increase in protein percentage. Increases in K fertilization did not affect percentage of protein in the tubers.

Results of a number of experiments in Russia show that increasing amounts of complete fertilizers increased the protein content of the tubers (Tikhonov and Avdeev 1970); long term fertilization with N-P-K increased the level of protein as well as essential amino acids with 75 to 90 percent of the essential amino acids being bound to the proteins (Tikhonov and Bychkov 1969A); the content of each individual essential amino acid in tubers was increased by fertilization. Yield of protein per hectare was increased three-fold by N-P-K- plus manure over the unfertilized but the biological value of the total protein in the potato was lower (Tikhonov and Bychkov 1969B); sulfate form of potash in the fertilizer produced more protein and improved the organoleptic rating compared with those grown with muriate form of potash (Zhuk and Gupalo 1970). Chelpanova (1972) obtained most marked increases in potato yield and of total N and protein N in the tubers from  $\text{NH}_4\text{NO}_3$  and urea forms of N. Highest protein content tubers resulted from N forms of ammonia water,  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{NH}_4\text{NO}_3$ .

In Sweden (Svensson 1969) application per hectare of 200 kg of N in the form of  $(\text{NH}_4)_2\text{SO}_4$  increased the content of N by almost one percent of the dry matter of the tubers above the application of 50 kg N per hectare.

Mica (1971) reported that in Czechoslovakia increased fertilization with N-P-K slightly reduced the total amino acid content. A level of 100 kg N/ha. gave the highest protein content. Lysine and leucine were the principal amino acids.

In Hungary a good N supply from  $\text{NH}_4\text{NO}_3$  increased the quality and the levels of total protein and non-protein N. Of the amino acids, glutamine, asparagine and arginine responded most to the N fertilization (Filep and Bukai 1969). In Bulgaria Dimitrov (1969) reported that N fertilization increased protein content of the tubers; in India Hukkeri (1968) found that N and P applications increased the percent crude protein in tubers and Coutrez-Geerinck (1970) in Belgium reported that in general the growing medium highest in N resulted in the highest amount of each amino acid in the tubers.

F. Photoperiod

Significant differences between day lengths in crude protein content of tubers both on a dry matter basis and a fresh weight basis were found with higher percentages under a 14-hour day than under continuous light (Umaerus 1970).

G. Effect of 2,4-D application

Spray application of 2,4-D to potato plants late in the growing season increased the protein content of Red McClure tubers grown in Colorado (Payne et al 1953).

H. Effect of application of sprout inhibitor to plants

Maleic hydrazide spray increased protein content of tubers immediately after harvest but after 60 days storage at 40°F it was lower than those untreated (Yasuda et al 1956); Rakitin and Strel'nikova (1970) report a decrease in protein nitrogen resulting from maleic hydrazide application.

I. Effect of application of herbicides

Simazine increased the crude and true protein content of potatoes (Mazur and Kawecka 1969).

J. Effect of gamma irradiation

Eight krad of gamma radiation had no significant effect on the digestibility or biological value of potato protein (Varela and Urbano 1971). Free amino acids increased gradually in proportion to the radiation dose up to 30 megarads (Boffi, Ferrari and Ferrara 1969). Irradiation of tubers with 7,000 - 30,000 rad which were then stored 15 days at room temperature increased by 30-50 percent the contents of free aspartic acid, proline, and aliphatic amino acids. Free glutamic acid and basic amino acids decreased slightly (Fujimaki, Tajima and Matsumoto 1968). Twenty-four hours after irradiation at doses up to 500 krad there was an increase in aspartic acid, asparagine, threonine, serine, alanine, leucine, isoleucine, lysine and arginine decreased (Kodenchery and Nair 1972).

K. Effect of other factors

Content of protein increased in virus X inoculated plant tubers (Chelolina 1969). Bordeaux mixture or copper chloroxide sprayed on plants at the beginning of flowering and 10 days later increased the protein content



of tubers (Gladilovich and Gudkova 1971).

The amount of amino acids was higher in a variety resistant to the attack of *Phytophthora infestans* (Merkur variety) compared to the less resistant variety (Bintje). Glutamic and aspartic acids comprised 22 percent of total amino acids in Bintje and 21 percent in Merkur before infection. Three days after infection, glutamic and aspartic acids comprised 7 percent of total amino acids in Bintje and 12 percent in Merkur. Serine and arginine increased after infection, especially in Bintje, where they increased from 11 percent of total amino acids to 29 percent (Olteanu and Brad 1969).

L. Methods and techniques used for determining protein and amino acids

Methods of preparing and analyzing potato samples for free amino acids are described on pages 357-361 of Talley et al 1964.

Kaldy et al (1972) measured the protein content of 21 varieties of potatoes grown under similar conditions by Kjeldahl N determination and by the binding of the dye Orange G. Desborough and Peloquin (1969) separated tuber proteins by acid gel disc electrophoresis. Peare and Thompson (1973) determined protein quality of a potato flour protein concentrate by rat feeding data and by microbiological assay using Streptococcus zymogenes.

Nutritive value per unit of land area and comparative nutritive values

Burton (1966) presents data for comparison of the nutritive values of potatoes to that of bread. In every case except that of calcium the values are based on the amounts of the various substances actually absorbed. The values for calcium probably are too high as some of it probably is unavailable both in potatoes and bread.

As a source of calories potatoes are, weight for weight, less than 1/3 as valuable as bread. However, if the potatoes are French fried or chipped they would be equivalent to or higher in calories than the same weight of bread. The boiled, steamed and baked potatoes are about as good a source of nitrogen as is the bread. Neither bread nor potatoes are a good source of vitamin A. Thiamine, riboflavin and niacin in boiled, steamed and baked potatoes are in amounts comparable to those in white bread, but much lower than those in whole meal bread. Bread contains no vitamin C, whereas the potato is a valuable source of this vitamin. Potatoes on the whole are inferior to bread as sources of phosphorus and calcium, but their iron content may be comparable or superior even to that of wholemeal bread.

Burton (1966) has compared the relative amounts of bread and potatoes which might be expected on the average to be produced per hectare in northern Europe. Four metric tons of wheat would result in about 4.8 tons of white bread (72% extraction flour) or 6.5 tons of wholemeal bread (92% extraction flour). Twenty-five tons of potatoes would give about 20 tons of peeled product. The comparative caloric values of the food produced from a hectare of land would thus be: potatoes 100; wholemeal bread 104; white bread 81. Twenty-five years ago potatoes outstripped wheat in efficiency of food production per area of land in that same area. In recent years wheat yields have increased relatively more than have those of potatoes.

MacGillivray and Bosley (1962) using average yield data of crops grown in the 1940's report that potatoes produced about five pounds per acre of the eight essential amino acids. This figure was about the same as those for wheat flour, corn meal, brown rice and carrots.

Thompson (1973) has presented more up-to-date data of yields and compared the net protein production of corn, wheat and potatoes in the United States. Potatoes produce a greater amount of net protein per hectare than does corn or wheat. He also points out that Borgstrom calculated one hectare of potatoes can supply the protein requirement for 9.5 people, while wheat can supply protein for only 6.3 persons.

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## II. POTATO QUALITY OTHER THAN PROTEIN

Specific gravity of raw potatoes has long been used as a measure of the texture of cooked potatoes. More recently specific gravity has been utilized as a forecast of the quality of processed forms of potatoes and also to a great degree the yields of the finished product. A very high correlation exists between specific gravity of raw potatoes, their total solids content and texture of the cooked potatoes. Although there are inherent inaccuracies of the specific gravity method of dry matter estimation, it is widely used because of the ease and rapidity of the determination and because no better method has yet been devised for large scale use.

It has been shown that tissue air space definitely influences specific gravity of individual samples. These variations could cause errors to as much as two percent of dry matter (Burton 1950). Other factors which may affect the relationship between specific gravity and texture of the cooked potatoes is the variable proportion of starch to other solids in the potatoes. Variations also may be caused by such factors as variety, growing conditions, areas of growth, internal composition of tubers, analytical techniques and perhaps others.

Fitzpatrick et al (1969) found that with 483 potato samples, representing breeding samples, five commercial varieties grown in six locations for two consecutive growing seasons and tubers grown in northeastern and north central sections of the U.S., the 95 percent confidence limits of the linear regression curve of specific gravity and total solids was  $\pm 2.11$ . These results show that equating specific gravity with total solids has limitations. For example, tubers with a specific gravity of 1.080 might range in total solids from 18 to 22 percent. The authors point out that the majority of points falling outside the confidence limits were from a set of samples (265 of a total of 483) whose total solids content was determined by a procedure considerably different from that used for the other 218 samples. The difference in technique was largely in the methods of drying the samples. Apparently there is no standard procedure for drying potatoes to determine their solids content.

Hundreds of papers from most areas of the world have been published showing the relationship between specific gravity of potatoes and texture of the



cooked potatoes and in many instances also indicating the close relationship between specific gravity and yields of some forms of processed products.

#### A. Carbohydrates

The constituents of the potato about which most is known are the carbohydrates, which are comprised largely of starch.

##### Starch

Starch comprising from 65 to 80 percent of the dry weight of the potato tuber, is calorically the most important nutritional component. In the raw tuber starch is present as microscopic granules in the leucoplasts lining the walls of the cells of the parenchyma tissue. The granules are on the average about 100 microns by 60 microns. Small grain size has been associated with small tubers, dry growing season, immaturity, potassium deficiency, and prolonged post-harvest storage. Properties of potato starch are determined fundamentally by the size of the granules.

It has been demonstrated repeatedly that there is a close correlation among specific gravity, total solids and starch content. (Von Scheele et al (1937) found correlation coefficients between specific gravity and dry matter content = +0.937; between dry matter and starch = +0.947). This close correlation is due to the fact that starch comprises a major proportion of the dry matter and that the percentage of non-starch solids in the fresh tuber is relatively constant, according to Burton (1966), around six percent. The method has obvious limitations due to differences in intercellular space, vacuolar content, etc. However, it provides a rapid method and often in commercial operations the only feasible method for making this important determination.

Some varieties inherently have higher starch contents than others. Factors which affect starch content of potatoes are fertilization, cultural conditions such as planting date, maturity, photoperiod, light intensity, soil moisture, spacing, soil and air temperatures, time of vine killing, presence of diseases, etc.

The relationship between starch content and texture of cooked potatoes has been investigated for about 75 years. It has been shown that there is a highly significant correlation between the starch content of the raw tuber and textural qualities such as mealiness, consistency, sloughing and sogginess. The properties of starch and the changes which they undergo during cooking must be considered in studies to explain variations in texture.

During the cooking process water is taken up by the starch granule which then starts to swell. In the range of 147° to 160°F, the starch begins to gelatinize. In potatoes of high starch content the cells tend to round off and separate as a result of the swelling of the gelatinized starch, resulting in a mealy texture. In potatoes of low starch content the cells retain their original orientation with each other, they do not round out and, therefore, result in a soggy texture. It is the amount of starch in the individual cell rather than the total amount of starch in the tuber that is related to the mechanism of cell separation.

Excessive cell separation, which quite often occurs in the cortical region of the tuber, results in sloughing.

Sugars - Potatoes may contain from zero to 10 percent sugar on the dry basis. Sugar content varies as to variety, degree of maturity, growing conditions, and storage temperature.

Potato breeders are intent on producing new varieties low in sugars, especially those to be used for processing.

The most important factor affecting sugar content of potatoes is the temperature to which they are subjected. At storage temperatures below 45-50°F., total and reducing sugars increase, the rate and extent of increase being greater the lower the temperature down to the freezing point.

The sugar content of potatoes is relatively unimportant except for those to be processed. Reducing sugar content of potatoes determines to a great extent the intensity of the browning reaction often resulting in excessive color development during processing and subsequent storage.

#### Non-starch polysaccharides

Relatively small quantities of the following occur in potatoes primarily in the cell walls and between cell walls of adjoining cells: (1) crude fiber, (2) cellulose, (3) hemicellulose, (4) pectic substances and (5) other polysaccharides. Some of these factors are related to texture of cooked and processed potatoes.

#### B. Lipids and organic acids

The average fat content of a potato is approximately 0.1 percent on a fresh weight basis, ranging from 0.02 to 0.2 percent. These small amounts of fat may be a factor in the oxidative deterioration of

dehydrated potatoes and flour.

A number of organic acids normally occur in potatoes although they are not related to nutritive value.

### C. Minerals

The potato is a good source of iron and magnesium as well as contributing some calcium, phosphorus, and most of the trace minerals that are lacking in milk. Potatoes are among the richest foods in potassium, but it is not known to be a mineral which is deficient in most diets. Almost all of the iron in boiled potatoes is present in an available form (McCance and Widdowson 1942). The intake of iron guards our bodies against anemia. About 10 percent of the iron in potatoes is lost in various forms of cookery. A substantial amount of iron is in the peeling of baked potatoes. Peeling potatoes resulted in a loss of 10 percent of the iron in boiled potatoes.

Although potatoes contain only a small amount of calcium, they have been shown to have a beneficial effect on calcium metabolism because they contain little of the phosphorus compound known as phytin.

Potatoes are very low in sodium and, therefore, are excellent in diet of those who attempt to reduce their blood pressure by limiting their intake of salt.

Potatoes are alkaline yielding foods in contrast to such foods as meat and eggs which yield an acid ash.

Magnesium has been given little attention in human nutrition, but it has become more important as research has shown that it will prevent and overcome the formation of stones in the bladder and the calcification of the soft tissues of the kidneys. It also is helpful in treating high blood pressure and difficulties with the heart. A typical person excretes about 200 mg of magnesium per day. To keep in balance this must be replaced and could be provided with about a half pound of potatoes if the remainder of the diet was low in this element. The human diets low in magnesium are those based largely upon milk or milk products because milk has only 12 mg. per 100 ml. (Halden 1956). It has only 1/5 to 1/10 as much magnesium as potatoes. Hence, milk and potatoes are excellent supplements since milk provides calcium and potatoes provide magnesium (McCay and McCay 1967).

The inorganic constituents of potatoes (extremes found)

mg. per 100 gm. dry basis		ppm dry basis	
P	43.0 - 605	Br	4.8 - 8.5
Ca	10 - 120	B	4.5 - 8.6
Mg	46 - 216	I	0.5 - 3.87
Na	0 - 332	Li	trace
K	1394 - 2825	As	0.35
Fe	3 - 18.5	Co	0.065
S	43 - 423	Ni	0.26
Cl	45 - 805	Mo	0.26
Zn	1.7 - 2.2		
Cu	0.6 - 2.8		
Si	5.1 - 17.3		
Mn	0.18 - 8.5		
Al	0.2 - 35.4		

From Lampitt and Goldenberg (1940).

### Vitamins in potatoes.

Of the six vitamins included in the recommended daily dietary allowances of the Food and Nutrition Board of the National Research Council, potatoes contain substantial amounts of four, namely ascorbic acid or vitamin C, niacin, thiamine, and riboflavin. Of the four vitamins, potatoes furnish Vitamin C in greatest amount.

### Vitamin C

Thousands of analyses of the vitamin C content of potatoes have been reported, the values ranging from 50 mg. in 100 gm. freshly harvested, immature potatoes to less than 10 mg. for potatoes stored for periods of many months.

In the United States potatoes contribute more vitamin C to the food supply than any other one food. One medium sized baked potato (100 gm) yield 20 mg. of vitamin C, which is 1/3 of the daily dietary allowance per man recommended by the Food and Nutrition Board of the National Research Council.

Variety, location, growing and storage conditions, degree of maturity, as well as methods of preparation for eating markedly affect the vitamin C content of potatoes.

### Variety

Hyde (1962) found ascorbic acid content of various potato varieties was 19 to 29.1 mg. per 100 grams. The content of vitamin C is higher in potatoes of varieties of more intense yellow flesh color. Lampitt et al (1945) found that in a number of varieties grown in England, the ascorbic acid values ranged from 16 to 41 mg. per 100 gm. when analyzed the day after harvest. Reestman et al (1943) also reported varietal differences in ascorbic acid content of potatoes grown in Holland.

### Location

It is not very clear as to the importance of location on the content of ascorbic acid in potatoes. Some differences which have been reported may have resulted from variation in factors other than that of location. Abramova (1961) reported that the amounts of ascorbic acid in a number of varieties grown in the Irkutsk region of the Soviet Union are higher than in those grown in the southern and western areas. In a study of eight varieties grown in various locations in New York with different conditions of fertilization, Karikka et al (1944) found no relationship of

location to vitamin C content of potatoes.

Ascorbic acid content of potato tubers increased with an increase in altitude at which the potatoes were grown in Russia (Blagoveschenskii 1937).

#### Soil and fertilizer

The effects of soil in which potatoes are grown on the vitamin C content of potatoes also is not firmly established. Biletska (1961) claimed that tubers grown on peat soil contained less ascorbic acid than those grown on mineral soils. Julen (1944) reported potatoes grown on sandy soil contained more ascorbic acid than those grown on heavier soils. On the other hand, Smith and Paterson (1937) found ascorbic acid content was not related to soil type and Karikka et al (1944) reported no variation in vitamin C in potatoes grown in a number of soils in New York.

Fertilization apparently plays a minor role in determining ascorbic acid content of potatoes. Smith and Gillies (1940) report that fertilizer application had no significant effect on the content of ascorbic acid. The results of Karikka et al (1944) were similar except that they found that with no nitrogen application there was a decrease in ascorbic acid content.

#### Degree of maturity

Several investigators have reported that immature tubers contained more ascorbic acid than mature ones (Smith and Paterson 1937; Woods 1935; Zilva and Barker 1939). Volkov (1959) found maximum content of vitamin C in tubers of early potatoes on the 27th day after the beginning of tuber formation. On the other hand Namek and Moustafa (1953) report that ascorbic acid content increases in potatoes until the tubers mature. A gradual decline follows after the vines start to dry up. Enachescu (1960) states that at maturity, tubers contain 20-50 percent more ascorbic acid than immature tubers. Some of these apparent differences in results could be due to the degree of maturity at harvest, the length of time between harvest and analysis of the sample, soil and air temperature immediately preceding harvest, methods of analyzing the samples, etc.

#### Storage conditions

During storage, ascorbic acid content of tubers decreases. This change is related to both length of storage and storage temperature. That ascorbic acid decreases when freshly dug potatoes are stored at 50 to 59°F. has been shown by a number of workers. At lower temperatures

ascorbic acid losses are even greater. At 41°F. ascorbic acid disappearance is greater than at 59° and at 32°F the losses are more rapid than at 50°F (Mayfield et al 1937; Rolf 1940; Karikka et al 1944; Murphy 1946). Olliver (1936), however, found slower rates of loss at 32° and 31° than at 50° or at room temperature.

Panalaks and Pelletier (1960) also found that tubers of Katahdin and Russet Burbank varieties stored at 68°F were higher in ascorbic acid than those stored at 40°F. Russet Burbank tubers stored at 40° and then held for three weeks at 68°F increased in ascorbic acid about 8-11 mg. per 100 gm. of tuber.

Ascorbic acid increases when potatoes, previously held at 50°F. or higher, are transferred to 32°, 34°, or 41°F (Kelly and Somers 1949; Barker 1950).

Greatest loss of ascorbic acid occurred during the first four months storage and were about the same as at nine months storage.

#### Effect of irradiation

Results on effect of irradiation on ascorbic acid in potatoes are somewhat conflicting. In general, gamma irradiation of potatoes soon after harvest causes considerable loss in ascorbic acid while irradiation a month or more after harvest causes little loss. Gamma irradiation at a dosage of 15,000 rep. resulted in a decrease in ascorbic acid content below the untreated for the first seven months storage at 38°, 45°, and 50°F. and for four months at 60°F. Beyond seven months storage there was very little difference between treated and untreated tubers (Sereno et al 1957). The rate of destruction of ascorbic acid by cobalt 60 irradiation is proportional to the dose administered. After four months storage, however, rate of loss from irradiated and nonirradiated tubers is about the same. Schwimmer et al (1958) found that ascorbic acid increases immediately after irradiation, but decreases after one day to the same concentrations as those untreated.

#### Effect of method of cooking and processing

Vitamin C is reduced during most methods of cooking and processing. Losses during boiling or steaming of peeled potatoes varies between 14 & 35%. In boiling unpeeled potatoes there is very little or no loss in vitamin C. Retention of ascorbic acid when potatoes are cooked with just enough water to keep them covered it is about 80 percent retention (Weits and Lassche 1960). The median value of biological available ascorbic acid is 11.5 - 13.5 mg. per 100 gm. for cooked new potatoes

and 1.7 - 2.9 mg. for cooked stored potatoes.

The highest loss, up to 50 percent, occurs in fried potatoes.

French fried potatoes lost 16 to 35% of the ascorbic acid while oven browned and hashed browned lost about 2/3 of the vitamin.

An extensive study of large scale cooking of potatoes was reported by Streightoff et al (1946). Their raw potatoes contained 16 to 27 mg. of ascorbic acid per 100 gm. Only 5% of this was lost in steaming while 24 to 68% was lost in mashed potatoes depending upon how long they were held after mashing. Baked potatoes lost 28% of the ascorbic acid and those which were boiled lost only 13%. The recommended daily dietary allowance for mature men is 60 mg. ascorbic acid as established by the Food and Nutrition Board, National Academy of Sciences - National Research Council (1968).

Losses in ascorbic acid which occur during various forms of processing potatoes will be presented further in section III.

### The B Vitamins

Potatoes contribute worthy amounts of three of the B vitamins, niacin, thiamine and riboflavin. The B vitamins as a group are essential not only for general health and growth, but for carbohydrate metabolism, smooth functioning of the nervous system, normal digestion and health of skin.

Streightoff et al (1946) found in raw potatoes in mg. per 100 gm., niacin, 1.7 - 3.3, thiamine 0.08 - 0.13 and riboflavin 0.03. There was little loss of any of the three vitamins (greatest loss was 17%) when potatoes were mashed, boiled steamed or baked.

Raw potatoes grown in Wisconsin showed niacin content of 1.54 mg. per 100 gm. Tubers stored at 40°F. increased in niacin content during the first month and then decreased to about the initial level after 6 months storage. Baked potatoes lost 4.2% of their original niacin content. A 100 gm. serving of boiled or baked potatoes supplies approximately 1/10 of the daily allowance for niacin (Page and Hanning 1963).

In India Choudhuri et al (1963) found thiamine content ranged from 0.09 to 0.110 mg. per 100 grams of potatoes. The median value for boiled potatoes found in Wisconsin was 0.082 mg. (Hanning and Mudambi 1962).



The recommended daily dietary allowance for mature men is 17 mg. equivalents niacin, 1.3 mg. thiamine and 1.7 mg. riboflavin as established by the Food and Nutrition Board, National Academy of Sciences - National Research Council (1968).

The following table presents the composition of 100 grams of raw and most cooked and processed forms of potatoes (Watt and Merrill 1963).

The effect of processing potatoes on the content of B vitamins will be presented in section III.

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### III. PROCESSED QUALITY OF POTATOES

Commercially prepared potato products are increasingly replacing potato products made in the home and restaurants from fresh potatoes. In the United States this trend is rapidly in progress and today over 50% of all potatoes eaten in that country are in processed form. In many other countries the trend is in the same direction and the percent of the total that is processed commercially extends from below 50% to zero in many countries. But I believe we should be looking into the future and anticipate that some form of potato processing will be utilized in essentially all potato producing countries in the world. Therefore, we should be interested in any changes nutritionally which may result from the various methods of processing potatoes. Murphy et al (1966) obtained nine potato products in one or more market forms which were analyzed, in most instances before and after cooking or other preparation. A home prepared form, with the exception of chips, was included in the analysis. Data in the following table show that there are no marked differences in food energy, protein or ash between the home prepared and commercially processed items.

Proximate composition of ready to eat potato products prepared from different market forms. (condensed from Murphy et al 1966).

Product	Food Energy cal/100 gm	Fat gms/100 gm	Protein gm/100 gm	Ash gm/100 gm	Carbohydrate (by difference) gm/100 gm
<u>Mashed</u>					
home recipe	77	0.5	2.2	0.97	16.2
Av. 4 brands					
dehydrated					
mix	83	1.3	2.2	1.17	16.1
<u>French fries</u>					
home recipe	224	8.8	4.9	1.90	32.9
Av. 7 brands					
frozen-oven					
heated	263	10.4	3.8	1.57	41.2
<u>Scalloped</u>					
home recipe	98	3.0	3.0	1.51	15.2
Av. 2 brands					
dehydrated mix	104	2.5	2.5	1.86	18.6
<u>Chips</u>					
Av. 4 brands	531	33.9	7.2	4.27	52.5

#### Proteins and amino acids

The content of several forms of cooked and processed potatoes in essential amino acids has been shown by Wertz et al (1956) and presented in the next table. In the study of data of this nature one must think in terms of foods with equal amounts of water or solid. For instance, boiled potatoes are about 80% water when eaten, bread is only about 35% water and potato chips are about 2%.

Amino acids of potatoes cooked in various ways and of white bread:  
mg per 100 gm.

Method of cooking	iso-leucine	leucine	lysine	meth-ionine	phenyl-alanine	threonine	tryptophane	valine	Total
boiled :	0.89	1.09	1.10	0.26	0.86	0.76	0.24	1.19	6.39
chips	3.46	4.21	3.66	0.92	2.79	2.48	0.57	4.22	22.31
French fry	2.89	2.25	2.03	0.47	1.57	1.47	0.45	2.48	13.61
mashed	1.21	1.54	1.31	0.35	1.04	1.02	0.22	1.25	7.94
white bread	5.51	7.94	2.54	1.62	5.29	3.32	0.89	5.46	32.57

From Wertz et al (1956)

Anisimova (1969) determined amino acids by paper chromatography in acid hydrolyzates of 12 varieties of fresh and cooked potatoes in Russia. Loss from cooking was 14-21%. Schwerdtfeger (1969) in Germany quantitatively determined 19 amino acids obtained by acid or alkaline hydrolysis from raw potatoes and from boiled and fried and from dumplings and salad. A significant decrease in total amino acid content occurred only with dumplings.

On dehydration of potatoes in West Pakistan least losses occur in protein and the highest in reducing sugar, while starch, sucrose and ash losses are intermediate (Bhatti et al 1968). Rios Iriarte et al (1972) using the biuret and Kjeldal methods of determination found the protein content of six potato cultivars ranged from 5.5 to 8.7 gm per 100 gm in potato flakes. Compared with that of the whole egg, threonine, lysine, histidine and tyrosine levels in potato flakes were about the same as whole egg; leucine, phenylalanine, arginine, isoleucine and methionine levels were higher in whole egg, but aspartic acid and glutamic acid levels were much higher in the flakes than in whole egg. With 5.28% potato flake protein in the diet, the essential amino acid levels supported vole growth. Protein efficiency ratios (PER) were lower than that for a casein diet. The lowest chemical score was found for methionine, ranging from 19 to 31 between the cultivars. With methionine-supplemented diets with the exception of cultivar 58, the vole weight gains attributable to supplementation were equal to or greater than the gains on the nonsupplemented diets. The apparent absorbability of potato proteins in unsupplemented and methionine-supplemented diets ranged from 55.3 to 63.8% and 69.5 to 88.6% respectively; that of casein was 64.5%.

Peare and Thompson (1973) prepared a potato flour concentrate by air classification techniques to remove the large starch grains. From the 13% whole potato flour they obtained a 33% concentrate comprising a highly nutritious protein. When fed in cooked form to weanling rats no differences were found in food

consumption or protein quality between whole potato flour and the concentrate derived from it. Based on protein efficiency ratios, nitrogen incorporation efficiencies (NIE) and weight gain measurements, the responses of rats fed potato diets as a percent of the responses of rats fed casein were 76, 74, and 68% respectively. The protein quality of the potato flours as determined by a microbiological method utilizing S. zymogenes was comparable to that determined by rat assay.

Kies and Fox (1972) report that methionine was the first limiting amino acid in dehydrated potato flakes for human nutrition. The mean N balances for seven human adults fed 4.0 gm. N per day from dehydrated potato flakes and 0.68 gm N from the basal diet were compared with those after supplementation with L-methionine, L-leucine, L-phenylalanine, or all three amino acids. The mean N balances were -1.18, -0.27, -0.83, -0.91 and -0.30 gm. N per day respectively. Subjects showed increase in N retention when methionine was used as a dietary supplement either singly or in combination with leucine or phenylalanine. Addition of purified L-methionine at a level equivalent to 0.37%, basis potato flakes, could significantly improve the protein nutritive value of the flakes. Sensory panel evaluation indicated the palatability was not adversely affected up to 1% supplementation.

Low specific gravity (1.065-1.075) Netted Germ potatoes lost about 40% of their total amino acid content by canning or chipping. Loss in drum dried flakes was about 20% and 4.5% in French fries. High specific gravity potatoes (1.095-1.106) showed a similar trend but the losses were much smaller. All processing methods reduced the available lysine content; chips and canned potatoes had the greatest loss followed by drum dried and French fried potatoes.

#### Vitamin A

Although this vitamin occurs in potatoes in very low amounts, it is possible to fortify processed products such as potato flakes with vitamin A. Cording et al (1961) showed that vitamin A content gradually decreased in dehydrated potato flakes in storage but was more stable when packed under nitrogen than in air.

#### Ascorbic acid

Watt and Merrill (1963) present as a year round average of ascorbic acid in raw potatoes 20 mg. per 100 gm. Dehydrated potatoes contain from 2.4 to 20.4 mg. per 100 gm. (Hanning and Mudambi 1962). Bring (1962) reported that reconstituted flakes contain approximately 1/4 as much vitamin C as raw potatoes and about 2/5-1/2 as much as fresh mashed potatoes. Total



ascorbic acid in raw potatoes, fresh mashed potatoes and reconstituted dehydrated potato flakes made in a commercial plant in Idaho was 29.3, 18.8, and 8.0 mg. per 100 gm. respectively in October; 11.7, 8.2 and 3.1 mg. per 100 gm., respectively in February; and 10.6, 6.8, and 2.8 mg. per 100 gm., respectively in May. Total ascorbic acid retention in fresh mashed potatoes compared with the raw potatoes was 64.0-69.7% on an "as served" basis or 75.1-77.6% on a dry weight basis. Total ascorbic acid retention in the reconstituted dehydrated flakes compared to the raw potatoes was 26.1-27.2% on an "as served" basis or 36.5-40.0% on a dry weight basis. Moisture content of the raw potatoes was 76.6%, in fresh mashed potatoes, 79.9% and in reconstituted dehydrated flakes 83.8% (Bring et al 1963). Ascorbic acid loss of dehydrated potatoes after two years storage at 41-82° F. in nitrogen was 29%; after three years it was 67%. No dehydroascorbic acid was found. Moisture and pH had no bearing on ascorbic acid values (Schillinger and Zimmerman 1965). Bring and Raab (1964) found that dehydrated flakes and dehydrated granules sampled in October of 1960, 1961, and 1964 contained respectively 8.0 and 6.6 mg. total ascorbic acid per 100 gms. sample as served. These same potato products sampled near the end of the processing season contained 2.8 and 2.1 mg. total ascorbic acid per 100 gms. moist flakes and granules, respectively. In general, total ascorbic acid retention in fresh mashed potatoes compared with raw potatoes in October and April was 51.7 and 47.4% respectively, on an "as served" basis. Total ascorbic acid retention in the reconstituted granules compared with raw potatoes in October and April was 25.3 and 18.0% respectively, on an "as served" basis.

Cording et al (1961) found that the level of ascorbic acid is maintained in dehydrated potato flakes at all storage temperatures for 28 weeks when the flakes are nitrogen-packed, but there are steady losses with air-packing.

Commercial brands of dehydrated potato products are different in their content of ascorbic acid. Fresh cooked potatoes contain 2 to 3 times more ascorbic acid than any of the cooked dehydrated unfortified potato products. Myers and Roehm (1963) stated that it would be well to fortify dehydrated potato products with ascorbic acid.

This is now being done. Dehydrated instant potatoes to which ascorbic acid has been restored have been added to the list of foods recommended for Federally-reimbursed school lunches under the National School Lunch Program.

One-half cup serving of reconstituted dehydrated instant mashed potatoes meeting the industry vitamin restoration standard is included with the foods listed by the USDA as good sources of ascorbic acid for meeting children's needs for this vitamin. The dehydrated product is fortified with approximately the same amount of ascorbic acid as that lost during processing. This amounts to about 20-mg. per 1/2 cup of edible portion.

Composition of potatoes and other foods (100 gms. edible portion).

Food	Water %	Food energy (cal.)	Protein gm	Fat gm	Total carbohydrate gm	Calcium mg	Phosphorus mg	Iron mg	Potassium mg	Vit A IU	Thiamine mg	Riboflavin mg	Niacin mg	Ascorbic acid mg
<u>Potatoes</u>														
Raw	79.8	76	2.1	0.1	17.1	7	53	0.6	407	trace	0.10	0.04	1.5	20
Baked in skin	75.1	93	2.6	0.1	21.1	9	65	0.7	503	"	0.10	0.04	1.7	20
Boiled in skin	79.8	76	2.1	0.1	17.1	7	53	0.6	407	"	0.09	0.04	1.5	16
Boiled, peeled	82.8	65	1.9	0.1	14.5	6	42	0.5	285	"	0.09	0.03	1.2	16
<u>Bread</u>														
White unenriched	35.8	269	8.7	3.2	50.4	70	87	0.7	85	"	0.09	0.08	1.2	trace
White enriched	35.8	269	8.7	3.2	50.4	70	87	2.4	85	"	0.25	0.17	2.3	"
Pumpernickel	34.0	246	9.1	1.2	53.1	84	229	2.4	454	0	0.23	0.14	1.2	0
<u>Beans</u>														
Red, raw	10.4	343	22.5	1.5	61.9	110	406	6.9	984	20	0.51	0.20	2.3	-
Red, cooked	69.0	118	7.8	0.5	21.4	38	140	2.4	340	trace	0.11	0.06	0.7	-
Mung, cooked	91.0	28	3.2	0.2	5.2	17	48	0.9	156	20	0.09	0.10	0.7	6
<u>Rice</u>														
White, unenriched cooked	72.6	109	2.0	0.1	24.2	10	28	0.2	28	0	0.02	0.01	0.4	0
* 2600 65 800 800 10 5000 1.3 1.7 17 60														

Selected from Watt and Merrill ( 1963 ) Composition of foods, raw, processed, prepared. U.S. Dept. Agr. Agriculture Handbook N° 8.

\* Recommended daily dietary allowances for male 35-55 years of age. Food and Nutrition Board, National Academy of Sciences - National Research Council. Seventh Edition 1968.

Total ascorbic acid content of raw potatoes, those freshly fried and those heated as frozen show string potatoes was 26.5, 43.2 and 27.6 mg. per 100 gm. soon after harvest and 12.2, 23.6, and 9.7 mg. per 100 gm. when processed after 5 1/2 months storage, respectively. Storage of frozen shoestrings up to 5 1/2 months does not significantly change the concentration of vitamin C (Bring 1966). During deep frying in oil vitamin C of potatoes decreased to 85% of that of the raw potato, which was 11.0-19.9 mg. per 100 gm. Vitamin C loss increases with time between initial frying and reheating (De Jongh and Tjolma 1961).

Vitamin C content in a variety of potato products ranges from 2.7 in potato powder to 20 mg. per 100 gm. in French fried potatoes (Kouwenhoven 1964).

#### B vitamins

Thiamine, riboflavin and niacin are relatively stable in dehydrated potato flakes under both nitrogen and air. Considerable losses in thiamine occur during processing because of the use of sulfite. Flakes fortified with a mixture of B vitamins and ascorbic acid and with a mixture of all the vitamins were considered unacceptable because of what was called "vitamin flavor" (Cording et al 1961). Thiamine, riboflavin and niacin were stable in dehydrated potatoes stored up to three years under nitrogen at 41-82°F even in opened containers which were then stored for two months in the dark in air (Schillenger and Zimmermann 1965). Hanning and Mudambi (1962) found the amount of thiamine in dehydrated potatoes fluctuates considerably from 0.004 to 0.292 mg. per 100 gm.

In canned potatoes the average value of thiamine is 0.036 mg. per 100 gm of drained product. This value is considerably lower than the median value found for boiled potatoes, 0.082 mg. per 100 gm. (Hanning and Mudambi 1962). Hentschel (1969) reports only a slight decrease in thiamine when potatoes are boiled, but a loss of 35-40% on frying. Riboflavin is lost to the extent of 5-30% in fried potatoes. Niacin decreases 25-30% with boiling but loses only 5-10% by frying.

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# **8**

## ***Outreach and Training Program***

## CONTENTS

	Page
PHILOSOPHY	375
STRATEGY	376
INITIAL CONCENTRATION OF EFFORTS	379
THE IMPLEMENTATION OF REGIONAL PROGRAMS	380
THE IMPLEMENTATION OF NATIONAL POTATO PRODUCTION PROGRAMS	382
SUMMARY	384

THE OUTREACH PROGRAM OF THE  
INTERNATIONAL POTATO CENTER (CIP)

An outline of its philosophy  
and strategy

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PHILOSOPHY

The basic goal of the International Potato Center is to raise the productivity of potatoes in countries of the developing world, where need and opportunity are greatest. Backed by a conviction that the potato has a much more important role to play in the feeding of the expanding populations of these regions, CIP and its donors have united in a program designed to maximize this potential contribution of the potato in Latin America, Africa, the Middle East and Asia. As this program evolves, the basic goal of the Center will ultimately be realized through CIP's Outreach Program operating in these regions and producing impact through national potato production programs.

The potential of the potato for helping to solve food problems received international recognition when the Consultative Group for International Agricultural Research included the International Potato Center in the network of centers being supported and funded by its donors. With such support the Center is challenged to deliver production-oriented research results quickly, and to translate this research into production break-throughs in developing countries. One of the principal measures of success for CIP will be increases in potato productivity in countries of the developing world. Another will be the increased utilization of the potato to help solve world food problems.

In order to translate research results into production break-throughs, the Outreach Program must work with national leaders to create the capacity in developing countries to utilize the technology developed by the Center. The Center is dependent on strong national programs to implement its strategy. In turn, the national potato programs will look to CIP for training and development of their leadership and personnel.

During the past two years, strategy for the Outreach Program has evolved and been defined so as to enable the International Potato Center to realize its goals. It is to be expected that continuing constructive discussions at all levels in the Center will examine the details of this strategic plan; and deter-



mine the most effective places for emphasis and the best route to follow. The strategy of the Outreach Program must remain flexible, responsible to needs of national programs and capable of communicating these needs to the Core personnel in establishing research priorities.

The goals of CIP will be successfully met only by a mobilization of the total available resources of the Center. A team approach, involving both Core and Outreach personnel working actively in the regional programs, is necessary to achieve the Center's objectives. It is the function of the Outreach Program to organize the regional structure through which research results can produce the impact on national potato productivity in countries of the developing world.

## STRATEGY

The fact that dramatically raising potato production in even one country is a complex and time-consuming task, means that CIP must concentrate much of its early efforts on a few carefully chosen objectives - a few specific national potato programs. Even though a world-wide program must gradually evolve that encompasses all the regions of the developing world, CIP does not have the personnel, budget, and, in many cases, the technology, to move ahead simultaneously on the entire front in all regions. Thus, specific objectives must be identified. In the identification process CIP must outline the general goals of a long-range Outreach Program. Then, within the context of this outline, those strategic national programs will be selected that give greatest promise for early impact and regional leadership. CIP has defined the following regions for its Outreach activities:

- I South America
- II Mexico, Central America, and the Caribbean
- III Tropical Africa
- IV Middle East and North Africa
- V Turkey, Iran, Pakistan, Afghanistan
- VI India (and Nepal)
- VII South-East Asia

Each of these regions represents a geographic unit that is cohesive for a regional approach. Each is in a particular stage of development, especially as far as the potato is concerned, and each has unique advantages and disadvantages that will directly affect the CIP regional efforts there. It is beyond the scope of this current statement to fully explore the potential of each region. However, following is a brief analysis of each region which is necessary to the next step of

selecting certain national programs for early concentration of effort.

## I. SOUTH AMERICA

(Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Perú, Uruguay, Venezuela)

This region is of particular interest to CIP because it is where the Center is located. The Center must make an early impact on national productivity in some country of this region, partly because it is the place of origin of the potato and partly because it serves as an early demonstration of how to attack the complex problem of raising national potato production.

Although the Center is located in Perú, CIP must consider its efforts to raise productivity in Perú similar to that of any other country. Perú does have the advantage of being close to the competence and research results provided by the CORE program of the Center.

## II. MEXICO, CENTRAL AMERICA, AND THE CARIBBEAN

(Caribbean Islands, Costa Rica, Cuba, El Salvador, Honduras, Guatemala, Nicaragua, Panama)

CIP's headquarters for this region is based in the Toluca valley of Mexico in association with a sister center CIMMYT (The International Center for the Improvement of Maize and Wheat). This regional program is a continuation of the former International Potato Program of the Rockefeller Foundation. At this regional location, CIP conducts a major portion of its late blight research and its international late blight trials. Resulting from these trials is a late blight germ plasm collection maintained for use by scientists of the world breeding for late blight resistance. The annual training for this region is CIP's oldest since it has an experience of over 15 years before becoming a part of CIP. Although its previous history was international, it's training now serves primarily the countries of Central America and the Caribbean.

### III. TROPICAL AFRICA

(Cameroon, Ethiopia, Ghana, Kenya, Malagasian Republic,  
Malawi, Nigeria, Tanzania, Uganda, Zaire, Zambia)

This regional program is associated with the Kenyan National Potato Program which has been assisted for a number of years by a team of scientists from the British Overseas Development Administration (ODA). For the past three years CIP has conducted a regional potato production course in cooperation with the Kenyan Ministry of Agriculture and the ODA team. Representatives from most of the countries of tropical Africa have participated in these training activities. A formal agreement has been concluded which permits the location of CIP's regional program in Kenya.

### IV. THE MIDDLE EAST AND NORTH AFRICA

(Algeria, Egypt, Iraq, Jordan, Lebanon, Lybia, Morocco, Saudi Arabia, Sudan, Syria, Tunisia, Yemen)

This regional program of CIP is based in Beirut at the Arid Lands Development Program (ALAD) which has an agreement with the Lebanese government for regional activities. This region was activated in 1974 with the location of a CIP production specialist at ALAD and the holding of a Seed Potato Production Seminar-Workshop in Cairo, at which representatives of most of the countries of the region participated.

### V. TURKEY, IRAN, PAKISTAN, AFGHANISTAN

Each of the countries in this region presents unique opportunities and problems of potato improvement. CIP expects to finalize the necessary agreements for an association with a country in this region and activate its regional program prior to the end of 1974.

## VI. INDIA

(India, Bangladesh and Nepal)

The potato has made tremendous gains in importance in India as a food crop in recent years. A scientific expertise exist within the Indian Potato Program which could be utilized by CIP in its regional approach. India already has a foreign technical assistance program for potato improvement in surrounding countries. Thus, CIP's participation in this region is unique and can be readily activated once agreements now in process have been finalized.

## VII. SOUTH-EAST ASIA

(Burma, Indonesia, Korea, Malaysia, New Guinea, Philippines, Sri Lanka, Thailand)

Within this region, several countries have had active potato improvement programs established. The Asian Vegetable Research Development Center (AVRDC) has indentified the potato as one of the important food crops for this region requiring its attention. Consequently, a working relationship has already been established by CIP with AVRDC scientists for a portion of CIP's regional interests. CIP is still in the process of identifying its regional program base.

## INITIAL CONCENTRATION OF EFFORTS

The identification of specific countries and the concentration of effort does not eliminate the possibility of CIP support and effort with any developing country. The concentration of CIP's initial effort will permit the early demonstration of CIP's capacity to produce production breac throughs and provide an example for similar successes in other countries. All interested countries are eligible for training programs and the appropriate exchanges of materials, and for visits by CIP personnel as justified.

## THE IMPLEMENTATION OF REGIONAL PROGRAMS

Regional Outreach Teams. As funds become available, CIP will staff its regional programs with three-man teams, based at the regional center of each of the seven regions.

The three-man team will consist of a training officer, seed production specialist and a production specialist working together to "create the capacity" within the developing countries of the region to utilize new technology and improved germ plasm made available through CIP.

### (1) Training Officer

Since any production impact of the CIP Outreach Program will be realized through national programs, CIP can most effectively assist countries which have well-trained personnel. It is the responsibility of the training officer to help identify people within a country who need further training to fit into the long range potato improvement plans of that country. In addition to this role in preparing nationals for positions of responsibility in their country's potato programs, the training officer will plan, coordinate and participate in regional "in-country" training courses.

### (2) Seed Production Specialist

Potato seed production is the area of a potato improvement program where the "scientist and the farmer meet". Research results can not have an impact on production without a well-organized seed program. A seed production program consisting of "basic", "foundation", and "certified" phases requires trained pathologists, knowledgeable of virus elimination techniques, sound storage technology and a distribution system capable of delivering potato seed in sufficient quantities to growers. An effective extension service is needed to demonstrate the value of using improved seed.

The seed production specialist has the responsibility of assisting national governments to develop seed improvement programs consistent with the country's needs and capabilities. Such a program could include the initiation of seed multiplication systems of the three categories of potato seed mentioned above (basic, foundation and certified) where appropriate, the development of a regulatory agency for the implementation of "seed laws", and the development of seed grower organizations.

(3) Production Specialist

Even in the developed countries, research results do not flow automatically from the scientist working at a university or research station to the cultivator who is the ultimate user of new technology. In developing countries which often lack an effective extension service, it is even more difficult to provide research results in a form which can be utilized by the farmer. The results of research obtained in the developed countries can rarely be transferred directly to a developing country and expected to have an impact on potato production in that country. There is a need for "adaptive research" which is adapting known technology to local needs.

The research scientist working with the CIP Outreach team must be a "production specialist" capable of working effectively with nationals to channel new technology into their experiments. Areas of responsibility would include:

- (1) the testing and distribution of germ plasm resistant to major diseases;
- (2) conducting of uniform and maximum yield trials; and
- (3) the study of potato storage problems, seed preparation and planting techniques and other agronomic practices. The adaptive research of a strong national program which is conducted with the aid of the CIP production specialist will also serve as a source of training for scientists from other countries of the region.

While the CIP regional team can stimulate the adaptive research necessary for national program development, there remains the vital step of extending this technology to the farmers' fields and production impact. In developing countries, this extension role is often sharply separated from the research group. This is regrettable, but a fact. Wherever possible, the CIP Outreach personnel will attempt to organize a smooth flow from adaptive research to production results in farmers' fields, utilizing all available research and extension personnel and institutions, and try to unite them in one national potato production effort. The task may be monumental in some countries, but this route is considered more desirable than establishing two separate thrusts in national programs, one for adaptive research and one for extending this research to achieve an impact on national production.

## THE IMPLEMENTATION OF NATIONAL POTATO PRODUCTION PROGRAMS

To achieve its stated objectives of raising potato productivity in developing countries, CIP must have a clearly defined plan of action. Assisted by CIP, a long-range plan for potato improvement must be developed by the nationals of each country. The plan must include a staffing pattern to provide trained personnel for the development of a national potato program. The staffing needs must be clearly outlined at the outset so the trainee will fill a specific role in his country's potato program upon completion of his training. The essential steps in the implementation of national programs can be summarized as follows:

- (1) Contact with national institutions responsible for potato production and determination of the extent of national commitment to potato improvement.
- (2) Identification of the responsible agency and personnel of this national institution through which the national potato production program is administered.
- (3) Organization of a national potato production scheme in a unified effort, utilizing the existing resources of support and personnel. The staffing pattern must be realistic, within the support possibilities of the country involved, and designed to meet the needs of the potato producing areas.
- (4) Assist national leaders to clearly identify deficiencies within their program and to develop a plan to satisfy essential program needs.
- (5) Initiation of the training programs to provide the personnel needed to staff the long-range national program.
- (6) Maintain contacts with trainees after they return to their national programs.

## STAFFING PATTERN OF CORE AND REGIONAL PROGRAMS

The following is a suggested staffing pattern and designation of certain

national institutions

national institutions

national institutions

national institutions

national institutions

national institutions

national institutions

CORE STAFF Lima/Mexico

Responsibilities

Richard T. Wurster	Head, Dept. of Outreach and Training	General Administration of Outreach Programs
John S. Niederhauser	Consultant to CIP Administration	all regions
James E. Bryan	Seed Production Specialist	advisor to National Programs

REGIONAL STAFF

<u>Region</u>	<u>Base</u>	<u>Regional CIP Representative</u>	<u>Impact Countries</u>
I. South America	Lima	Oscar Hidalgo	Peru, Brazil, Chile, (Ecuador, Bolivia, Colombia)
II. Mexico, Central America, Caribbean	Mexico	Manuel Villarreal	Guatemala, Costa Rica, Cuba
III. Tropical Africa	Nairobi	-----	Kenya, Nigeria, Ethiopia
IV. Middle East and North Africa	Beirut	Primo Accatino	Lebanon, Syria, Algeria (Egypt-training center)
V. Turkey, Iran, Pakistan, Afghanistan	(-----)	-----	Turkey, Iran Pakistan
VI. India	Simla	-----	States of Punjab, Uttar Pradesh, Nepal, Bangladesh
VII. South-East Asia		-----	Sri Lanka, Indonesia



## S U M M A R Y

This is a presentation of CIP's philosophy and strategy to make early and definite progress towards its basic goal of increasing potato productivity in countries of the developing world. In its strategy CIP will concentrate its initial efforts primarily in specific national potato production programs, and demonstrate a successful impact there. This should lead to requests for similar assistance to accomplish production break-throughs in other national potato programs.